The effect of plant-based mulches on soil properties that influence crop yield

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DECLARATION

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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LIST OF ABBREVIATIONS

ADF – Acid Detergent Fibre

ADL – Acid Detergent Lignin (lignin)

AMF - Arbuscular Mycorrhizal Fungi

$B_{(12)}$ – Biomix (12 plant species)

$BL_{(12)}$ – Biomix long

$BS_{(12)}$ – Biomix short

C - Control

d.a.m.a. – days after mulch application

GS – Growing stage of barley plants

$H_{(17)}$ – Herbal (17 plant species)

$HL_{(17)}$ – Herbal long

$HS_{(17)}$ – Herbal short

NDF – Neutral Detergent Fibre

$P_{(1)}$ – Perennial rye grass (1 plant species)

PCA - Principal Component Analysis

$PL_{(1)}$ – Perennial rye grass long

PLFA - Phospholipid fatty acid

$PS_{(1)}$ – Perennial rye grass short

$S_{(6)}$ – Smart grass (6 plant species)

$SL_{(6)}$ – Smart grass long

$SS_{(6)}$ – Smart grass short

W – Wood chips

WHC – Water holding capacity

Abstract

Plant-based mulches can improve soil physical conditions. In addition, shredded mulches of high quality are shown to enrich soil nutrients more than those of lower quality in a short term. However, the effect of size and quality of diverse mulches on soil nutrients and soil microbiology at later stages of decomposition has not been properly investigated. I hypothesized that, at later stages of decomposition, long size diverse residue mulch maintains higher fertilization capacity than short residues, and residue quality affects soil microbiology due to changes in decomposition rates, influencing soil nutrients and crop quality, but not crop yield.

Two rhizotron and one field experiments were conducted to evaluate the effect of mulch residue size (<1.5 cm vs >30 cm) and diversity on soil chemistry and microbiology at later stages of decomposition, and on crop yield, crop quality, and residue quality at the end of the growing season. Residue mixes of 17, 12, 6, and 1 plant species, wood chips and control treatment with no residues were used.

Residue biomass loss was not affected by its size, but it was higher in residues of higher quality. *Long residues* retained higher fertilization capacity than *short residues* at harvest. Residues of lower quality had higher fertilization capacity than those of higher quality at a timepoint long after harvest. In field, the remaining residues mixed with crop residues at harvest produced residues of higher quality than the crop residues alone. Residue quality affected crop quality but not yield. Soil nutrients were affected by both residue size and residue quality. There were indications that residue quality affected soil microbial biomass, at later stages of decomposition, although differences were not significant, and Arbuscular Mycorrhizal fungi root colonization was increased with increasing residue plant species richness.

Generally, mulches of diverse residues can maintain soil fertility throughout cultivation period. Soil chemistry can be affected by both residue size and quality, and possibly soil microbiology can be affected either by residue quality or by residue diversity. Further investigation is needed with continuous applications of mulches to evaluate their long-term benefits.

Chapter 1

Introduction

1. Soil fertility, inorganic, and organic fertilizers

Soil fertility is an important factor that influences both quantity and quality of agricultural production and it involves appropriate management of essential nutrients for plant growth (ORC, 2016). Improvement of all soil physical, chemical, and biological properties is necessary to maintain high soil fertility (Maeder *et al.*, 2002). The term Green Revolution was introduced in 1960s to describe the potentialities from the use of new technologies in the increase of agricultural production, achieved by researchers who produced new cereal varieties. However, these new varieties were dependent on artificial inorganic fertilizers and plant protection agrochemicals (Briggs, 2009). Artificial fertilizers used to supply soil nutrients are costly, and they deteriorate soil fertility by reducing soil microbial activity (Nakhro and Dkhar, 2010). In addition, they can cause serious environmental pollution (Rodrigues *et al.*, 2013). Discharge of large quantities of phosphorus in intensive agriculture or effluents of livestock farms into surface water bodies (lakes or rivers) stimulate water eutrophication that impact ecosystem and human health (Liu *et al.*, 2020). NO_3^- is leached downwards and pollutes groundwater reservoirs causing human health risk (Zhang *et al.*, 2021). NO_3^- leaching is lower for organic than for inorganic fertilizers because the solubility of N in soil solution is lower due to enhanced denitrification, and higher capture of N in soil organic matter (Jalota *et al.*, 2018). Moreover, inorganic fertilizers result in disturbance of the ratio of C supply in soil in comparison to other nutrients at the expense of soil microbial growth, which triggered research on organic fertilizers as an alternative to solve these problems (Lotter, 2003).

Several forms of organic fertilizers have been proposed, especially in organic farming systems but also in conventional farming, to promote soil fertility (nutrient supply, microbial activity, and soi physical conditions) (Maeder *et al.*, 2002). The fermentation process of composting organic materials is considered to produce high quality fertilizers, free of pathogens and phytotoxins (Chang *et al.*, 2021). However, the process of producing compost is dependent on organic material quality, microbial structure, and environmental conditions (Covino *et al.*, 2016). Composting takes time, and it requires special treatment. Alternatively, the use of cover crop or cash crop residues without fermentation is much easier. Plant residues can be either incorporated into soil or applied as mulch. Traditionally, residues incorporated into soil were believed to undergo a faster and more complete decomposition than when applied as mulch. However, research showed that this is dependent on residue quality. Indeed, Abiven and Recous (2007) found that incorporated residues initially were decomposed faster than those applied on the soil surface, but the results were similar with those in mulch application after a few days or even further, net N mineralization was higher in mulch application than in incorporation, when residues had low C:N ratio.

2. The role of mulch in soil fertility

Mulch has been defined as ‘’an organic material used to spread over the surface of the soil to prevent evaporation or erosion, e.g. dead leaves or straw’’ (Bateman, Curtis and McAdam, 1963). Mulch may contribute to maintenance of soil fertility by affecting soil physical, chemical, and biological properties.

2.1. Soil physical properties

In recent decades, research in conservation tillage systems highlighted the advantages of mulch application in agricultural practice. In conservation tillage, minimum soil disturbance, continuous soil cover with organic material, and a high diversity of crop species are sought (FAO, 2015). Undisturbed soil and soil coverage with plant-based mulch is considered to meliorate soil physical conditions. Mulch reduces water evaporation and increases water infiltration (Sharma et al., 2011). It affects significantly soil water content, mainly in 0-30 cm depth (Su et al., 2014). However, in dry regions with no irrigation usually the available plant biomass for mulching during summer is insufficient to reduce evaporation. Mulumba and Ral (2008) estimated that mulch rate of 8 Mg/ha was optimum for sufficient water retention and aggregate stability. Mulch alleviates the effect of mechanical soil compaction (Siczek & Lipiec, 2011). Jordan et al. (2010) in an experiment with straw mulch in semi-arid conditions noticed a decrease in soil bulk density and an increase in aggregation stability, soil porosity, and water holding capacity.

Mulching reduces soil erosion from air considerably as well as water run-off but in a less extent. Protection from soil erosion is dependent mostly on mulch thickness, while that from run off is affected greatly by mulch cover (Prats et al., 2017). Different plant species demonstrate variation in their capability to protect from soil loss in different rain intensities and rates of mulch application (Donjadee & Tingsanchali, 2016).

Mulching mitigates the effect of very high or low temperatures and reduces daily temperature oscillation (Blanco-Canqui and Lal, 2009). This regulation effect in soil temperature is an advantage in hot areas or cultivation periods but may be a disadvantage in wet regions where it may prevent an early planting or sowing. In an experiment, mulching resulted in lower yield, attributed to lower temperature that affected the early establishment of the seedlings (Halde et al., 2015). However, in another experiment, rice straw mulch enhanced biomass accumulation of oil seed rape during the seedling stage. This enhancement was attributed to maintenance of short temperature range and conservation of water availability, irrespective of water supply (Su et al., 2014).

2.2. Soil chemical properties

Plant-based mulch is a sustainable way to enrich soil nutrients because it can be produced on the farm as cover crop and cash crop residues. In addition, mulch is a low-cost fertilizer, provided it is locally produced, and is more easily applied than compost. However, there is still a lot of research to be

conducted on the effects of mulching on soil chemical and biological properties considering the complexity of their interactions.

Much research has looked at the effect of mulch in C and N mineralization (Dietrich *et al.*, 2017; Castro and Whalen, 2016; Wayman *et al.*, 2015). The amount of C mineralized is dependent on the amount of residues applied, but N may be subjected to immobilization in N deficient soil (Dietrich *et al.*, 2017) or when the C:N ratio of plant residues is very high (Siczek and Lipiec, 2011). N mineralization is dependent on residue type and is significantly higher in residues with a low C:N ratio and high N content (high quality residues), as is the case of legumes (Wayman *et al.*, 2015). A C:N ratio equal or less than 24 is considered to lead to net N mineralization because in average soil decomposer microbes usually have a C:N ratio of about 8 and they can assimilate about 1:3 of the residue C they metabolize (Brust, 2019; Hadas *et al.,* 2004), although this threshold can be higher if soil fauna with C:N ratio more than 8, that are fed on decomposer microbes, are involved in the decomposition process (Frouz, 2018). However, in later stages of decomposition this trend seems to be reversed and residues of lower quality are decomposed faster than those of higher quality. Probably, this happens because the easily decomposable compounds of the higher quality residues are initially decomposed faster than those of the lower quality residues, due to higher N availability (Angers and Recous, 1997; Bremer, Houtum and Kessel, 1991), but N restricts the decomposition of lignin at later stages due to microbial competition (Fog, 1988). Decomposition rate is decelerated over time, while the content of phenolic and aromatic C increases (Xu et al., 2017).

Residue C:N ratio affects decomposition greatly in N-poor soils, while its effect in N-rich soils can be insignificant (Bonanomi et al., 2017). The C:N ratio varies, depending on the residue plant species, on the plant tissues, and on the growing stage of the plants. As a plant grows to maturity its C:N ratio as well as its lignin and cellulose content increase. Generally the foliage has lower C:N ratio and lignin content than stems and roots (Singh & S. Khind, 1992). In N-poor soil, N-deficiency symptoms in plants may occur soon after mulch application but they subside as decomposition proceeds (Siczek and Lipiec, 2011).

An increase in soil C may take over 9 years to be detected (Olson *et al.,* 2010; Peterson *et al.*, 1998). By contrast, soil available N is subjected to the processes of nitrification and denitrification and its concentration is altered dramatically from time to time mainly due to NO_3^- leaching (Calderón *et al.*, 2016). As decomposition proceeds, soil NH_4^+ content is reduced in favour of NO_3^- due to nitrification (Castro and Whalen, 2016). Rodrigues *et al.* (2013) found increased contribution of N-rich legume cover crops in total soil inorganic N in a year after mulch application in comparison to natural vegetation, but also higher loses of N supply due to higher NH_3 volatilization rather than NO_3^- leaching. Nutrient losses in mulching are mainly due to volatilization and water run-off. In contrast, when plant residues are incorporated into soil their decomposition is more rapid and nutrient loss is mainly due

to leaching. Ammonia volatilization is favoured not only in mulching but also when the soil has high pH and low Cation Exchange Capacity (Singh & S. Khind, 1992).

Apart from the C:N ratio, other factors have been found to affect decomposition, such as N:P ratio, lignin, cellulose, and polyphenol content of residues (Xu *et al.*, 2017; Zhonglu *et al.*, 2015; Vahdat et al., 2011). Hemicellulose and cellulose are considered intermediately decomposing compounds, and lignin, tanins, and polyphenols are considered slowly decomposing compounds, while soluble carbohydrates and amino acids are decomposed rapidly (Hadas *et al.,* 2004). Therefore, residues of high quality are those with not only low C:N ratio and high N content but also with low recalcitrance (hemicellulose, cellulose, and lignin-like compounds).

Legumes are both N-mobilizing species due to their N fixation capacity (Finney *et al.*, 2016) and P-mobilizing species (Jeffery *et al.*, 2018). For that reason they are extensively used as alternative fertilizers (green manure) (Li *et al.*, 2014), but other plant species also demonstrate nutrient-mobilizing capacity (Lambers *et al.*, 2006). Nutrient-mobilizing plant species can mobilize unavailable nutrients from the soil in conditions of nutrient deficiency (Li *et al.*, 2014), but we know little about their use as mulch or their effects on soil. P-mobilizing plant species can either hydrolyse organic P or solubilize inorganic P fixed into soil by excreting phosphatases, protons (H^+ excreted mainly in alkaline soils), and carboxylates (Li *et al.*, 2014). Topsoil foraging plant species are advantageous in P acquisition as large quantities of insoluble P is fixed in topsoil (Lynch and Brown, 2001). Mulching contributes to maintenance of high level of available P for plant uptake in comparison to natural succession processes where stable P increases over time (Oelmann *et al.*, 2017). Graminaceous monocotyledonous plant species and species with cluster roots are capable to mobilize Fe and Zn in deficient soils (Li *et al.*, 2014). There is a gap in research on mineralization of nutrients other than C, N, and P and the role played by soil microbes. The amount of available nutrients is dependent on the number of mulching applications. Pavlu et al. (2016) observed higher concentrations of extractable P and K in ascending order from one to three applications.

2.3. Soil biological properties

Mulch application composed of large residue fragments decreased N availability to soil microbes in comparison to short residues, favouring decomposers of higher C:N ratio materials such as fungi at the expense of bacteria (Giacomini *et al.*, 2007). Residues with higher C:N ratio favor fungal growth more than bacterial. The decrease of soil pH has the same influence. (Grosso *et al.*, 2016; Barreiro *et al.*, 2016). However, in a long term, mulching application seems to favour bacterial growth in all cases. In a three-year experiment, bacteria dominated the soil microbes' community from the beginning in high quality residues. In low quality residues fungi dominated only the first year and bacteria the rest of the period (Frasier et al., 2016). Fungi are more competitive than bacteria in the decomposition of coarse residues or mulch due to colonization of residues by hyphae which, in addition, can transfer nutrients

between soil and residues (Ambus and Jensen, 1997). However, fungal growth was found to be negatively correlated with phenolic compounds in decomposed litter (Chomel *et al.*, 2014). Fungi and gram-positive bacteria are favoured by low soil moisture content, while high soil moisture facilitate the growth of gram-negative bacteria (Chen *et al.,* 2020). Gram-negative bacteria preferably exploit the more easily degradable newly added litter, while gram-positive bacteria are more efficient in degradation of more recalcitrant or older organic substrates (Fanin *et al.,* 2014). An increase in fungal: bacterial ratio and potentially in soil microbial biomass is an indication of a shift toward an ecosystem more reliant on biological functions (Bardgett & McAlister, 1999; Yeats et al., 1997).

Other soil organisms than bacteria and fungi are also involved significantly in nitrogen mineralization from plant residues. The most important of them belong to nematodes, protozoa, and earthworms. According to a metanalysis, the effect of soil fauna to litter decomposition as mulch is affected by the residue C:N ratio. It is significant at C:N ratio >30 and higher at C:N ratio between 20 and 30. At C:N <20 no significant effect was observed (Frouz et al., 2015).

Mulching contributes to the control of soil-borne diseases and has a significant influence on predation pressure. Long residues of higher quality terminated with a roller crimper (whole plants) were found to promote higher predation pressure than residues of lower quality applied as green manure (fragmented plants) (Magagnoli et al., 2018). Mulching also favors earthworm activity in comparison to incorporated residues (Calderón et al., 2016).

However, important issues such as weed control still remain considerable challenges (Wayman *et al.*, 2015). It has been shown that weed emergence can be reduced by mulch applied in sufficient amounts, more than 10 Mg/ha (Ranaivoson *et al.*, 2018). Weeds can be controlled when a high biomass production plant is used to provide residues and is combined with crop varieties with resistance to weeds (Randrianjafizanaka et al., 2018).

3. Residue size

Decomposition rate and N mineralization are also affected by the residue size, but they are highly dependent on residue C:N ratio and on soil N concentration. In residues with a very high C:N ratio, decomposition rate was higher in residues of larger particle size than those of shorter one after 17 days of incubation, while in residues with very low C:N ratio this happened after 65 days (Angers and Recous, 1997). Jensen and Ambus (1994) in a field experiment showed that N immobilization in residues with high C:N ratio was not affected by residue particle size when soil had low N content, probably due to N limitations. By contrast, in residues with low C:N ratio those with smaller particle size resulted in higher CO_2 emissions, indicating higher microbial activity, but only for the initial short-term period. On a long term the nitrogen dynamics were similar for ground and coarse residues (Ambus *et al.*, 2001). This is important because it shows that the residue particle size does not affect the final amount of nitrogen that is released in the soil but only the time period of the release and

availability to plants. Most research on residue degradation is focused on fine or fragmented residues, usually less than 2.5 cm, incorporated into soil, rather than on whole plants applied as mulch, probably because short residues incorporated into soil are decomposed faster than long residue mulch at the early stage of decomposition where nutrient fluxes are more intense (Su *et al.,* 2014).

The type of residue fragmentation, vertical or horizontal, does not seem to affect significantly the release of nutrients from decomposition (Reichert et al., 2015). Smaller residue particle size favour bacterial than fungal growth due to increased contact surface between residues and soil (Barreiro, et al., 2016). Residue particle size, soil disturbance and climatic conditions are important factors of nitrogen mineralization rate. Drying residues, larger particle size, lower temperatures and mulching application contribute to lower nitrogen mineralization rate in contrast to incorporated into the soil chopped residues, under higher temperatures in optimal moisture conditions (Singh & S. Khind, 1992).

4. The effect of mulch on crop yield and quality

The residue type and its particle size likely affect crop yield and quality. A rapid decomposition is not always beneficial, as it may provide nutrients to a crop early but may also deprive it from latter nutrient availability. The decomposition of *Phacelia tanacetifolia* as a mulch was more rapid in a low Monthly Aridity Index year, resulting in a reduction of a successive tomato yield (Marinari et al., 2015). The effect on yield is controversial, while that on crop quality seems to be more obvious. Straw mulch increased winter oilseed rape yield regardless of the level of N supply, probably due to improvement of soil water and temperature conditions (Su *et al.*, 2014). Yield of maize was reduced with increasing residue C:N ratio, while it was increased with increasing residue N or soil C content (White *et al.,* 2017). Rodrigues *et al.* (2013) observed slightly higher olive yield in field plots of legume cover crop mulch than in those of natural vegetation, but the differences were not statistically significant. By contrast, N concentrations in the olive pulp was significantly higher in the legume mulch plots in comparison to the natural vegetation plots. In another experiment, mulch increased seed protein, carbohydrates, P, N, and ash content, but did not improve yield in comparison to artificial fertilizer (Awopegba *et al.,* 2017). Squash yield was increased in saline soil under rye straw mulch in comparison to a control (Abd El-Mageed et al., 2016). A positive effect of mulch on yield and protein content of soybean was also reported by Siczek and Lipiec, (2011). Residue fragmentation affected the decomposition rate but did not demonstrate significant influence on crop yield, although the release of macronutrients N, P, K, Ca, and Mg was higher in residues with smaller particle size (Reichert et al., 2015).

5. Mixtures of plant residues

Mixtures of plant species either cultivated as cover crops or applied as mulch are considered to provide multiple ecological services. Cultivation of a combination of legume and non-legume species has been suggested to both provide sufficient amounts of available N after cover crop termination (green manure effect) (Tribouillois *et al.*, 2015) and avoid NO_3^- leaching during cultivation (catch crop effect)

(Justes *et al.*, 2012), respectively. However, in any case NO_3^+ leaching after cover crop termination is lower from residues with high C:N ratio than from those with low C:N ratio, or when residues are applied as mulch, rather than incorporated into the soil (Ambus et al., 2001). This probably happens because N supplied by residues must be first mineralized before it leaches (Singh and S. Khind, 1992) and mulch results in slower decomposition and N mineralization rates than the incorporation of residues into the soil. The difference is higher in residues with a wider C:N ratio (Bremer *et al.,* 1991).

Another important ecological service of mixtures of plant species is their contribution to ecosystem stability by increasing soil microbial diversity (Wu *et al.,* 1993). Soil microorganisms demonstrate enzymatic specificity in decomposition of diverse substrates varying in chemical composition (Fontaine *et al.,* 2003; Zvyagintsev, 1994). However, it seems that environmental conditions also play an important role in this relation. Calderon et al. (2016) failed to find any positive influence of cover crop residue diversity on soil microbial structure in a semiarid environment, suggesting that it takes longer time to observe a positive effect in such conditions. The impact of this relation between residue and microbial diversity on ecosystem stability is an important reason that justifies further investigation on the use of cover crops of mixtures of plant species rather than single plant species.

Litter species composition and associated functional traits seem to be more conducive in the soil microbial community structure rather than litter species richness (Santonja *et al.*, 2018). However, cover crop species richness has been found to increase sporulation of symbiotic Arbuscular Mycorrhizal Fungi (AMF) colonizing the cultivated cover crop plant roots, although this effect was found in N-limited soil conditions (Burrows and Pfleger, 2002). More research is needed to investigate the effect of diverse residue mulch on AMF root colonization of cash crops and on other symbiotic relations.

6. Mulch handling

In field conditions, residue mulch is normally applied heterogeneously (in patches rather than evenly distributed on the soil surface). This may result in a smaller soil microbial population due to slow diffusion of soil N into the decomposition area during the first months of decomposition, more pronounced in residues with high C:N ratio. The rate of microbial activity, measured by respired CO_2, was initially slower but it gradually became higher in inhomogeneous than in uniform distribution of residues. The two rates tended to be equal in the end (Magid *et al.*, 2006). It may be easier for plant roots to have access to soil nutrients when decomposable material is gathered in patches (Bonkowski *et al.,* 2000).

In fields where residue mulch is applied, the phenomenon known as the 'plant legacy effect' is likely to take place, according to which, the decomposition of recently applied residues can be affected by the quality of previously applied residues (Zheng and Marschner, 2017). Residue C:N ratio seems to play an important role in legacy effect. Nutrient availability was higher when the C:N ratio of the first

residue was lower (Nguyen *et al.*, 2016). Further investigation is needed to detect the influence of the legacy effect on soil microbial activity and nutrient mineralization in continuous applications of residue mulch.

Aims and hypotheses

The overarching aim of my research was to assess the effect of size, quality, and plant diversity of mulch on temporal fluxes of soil nutrients, concomitant changes in microbial activity, resultant residue quality, and finally on crop quality and crop yield, with a view to applications in both organic and conventional farming systems.

The aim of experiment 1 was to investigate the optimal size of mulch residues (whole plants vs comminuted) and the role of plant residue diversity and quality to gain benefits from decomposition. Short residues are expected to decompose faster at the early stage of decomposition than long residues due to higher contact with the soil surface, and this trend may be reversed at the later stages of decomposition (Ambus *et al.*, 2001; Angers and Recous, 1997). However, decomposition is decelerating over time (Ostrofsky, 2007), and thus it is possible that the long residues maintain higher fertilization capacity than short residues at crop harvest. Likewise, residues of higher quality (lower C:N ratio, higher N, and lower recalcitrant substances) are initially decomposed faster than residues of lower quality, and this trend may be reversed at the later stages of decomposition (Bremer *et al.*1991;). Residue diversity (plant species richness or plant species composition) has been found to promote soil microbial diversity, but the effect of residue diversity on residue decomposition is either synergistic or antagonistic or additive depending on chemical composition of residues (Redin *et al.*, 2014; Hattenschwiler *et al.*, 2005). Arbuscular Mycorrhizal Fungi (AMF) spore number and volume were found to increase with the increase of crop residue species richness (Burrows and Pfleger, 2002). Previous research has shown that residues of smaller particle size may release higher amount of soil macronutrients in comparison to longer particle size, but crop yield may not be affected by residue size (Reichert *et al.*, 2015). The effect of initial residue quality and diversity on crop yield is controversial and often differences are not significant, while crop quality (including crop morphology) seems to be more readily affected (Rodrigues *et al.*, 2013; Awopegba *et al.,* 2017; Siczek and Lipiec, 2011). Therefore, I hypothesised that (i) *long residues* maintain higher fertilization capacity than *short residues* at the end of the growing season, and (ii) soil nutrient availability at the later stages of decomposition are affected by both residue size and residue quality (residue C:N ratio, N, and recalcitrant substances), (iii) AMF root colonization increases with increasing residue species richness, (iv) and crop quality is affected by both residue size and residue quality but with no deleterious effect on crop yield.

The aim of experiment 2 was to investigate the effect of initial quality and diversity of long size plant-based residue mulch on soil chemical and biological properties at later stages of decomposition as well

as on crop yield and quality. Based on results of both previous research, which have already been mentioned, and of the previous experiment, the effect of the initial residue diversity and quality on crop quality (including crop morphology), but not on crop yield, was expected to be significant. Moreover, a reverse in decomposition rate, which normally is higher in residues of higher quality initially and in residues of lower quality later, was expected to result in higher nutrient release, and possibly in higher soil microbial biomass by higher quality residues at earlier stage of decomposition than that of lower quality residues. Effect of residue quality on soil microbial biomass has already been reported in previous research (Grosso et al., 2016; Barreiro *et al.*, 2016). According to this reasoning, the remaining residues of lower quality at a timepoint long after harvest was possible to maintain higher fertilization capacity than residues of higher quality due to lower decomposition rate. Therefore, I hypothesized that (i) residues of higher quality (lower C:N ratio, higher N content, and lower recalcitrance) are initially decomposed faster than those of lower quality, but the rate of decomposition is reversed at later stages of decomposition, resulting in significant differences in soil microbial biomass, in nutrient dynamics, and in crop quality, but not in crop yield, (ii) the remaining residues at a timepoint long after harvest, at the end of the growing season, still maintain a fertilization capacity which is higher in residues of lower quality.

The aim of experiment 3 was to evaluate the quality of the remaining residue mulch at harvest, and to investigate the effect of quality and diversity of whole-plant residue mulch on soil chemistry and microbiology, and on cash crop quality in field conditions. Normally, during decomposition the C:N ratio of residues is reduced (Akratos *et al.,* 2017; Spain and Hodgen, 1994), and it is possible the mixture of the remaining residues with the crop residues at harvest to improve the quality of the crop residues. Moreover, previous research has shown that both residue diversity (Santonja *et al.*, 2017; Redin *et al.*, 2014) and residue quality (Leppert *et al.*, 2017), may affect soil chemistry, and soil microbiology, but usually the effect of residue quality is higher than that of residue diversity (species richness) (Santonja *et al.*, 2018; Leppert *et al.*, 2017; Ball *et al.*, 2009). However, in field conditions, but sometimes also in incubation experiments, soil microbial activity is not always related with soil microbial biomass due to existence of hotspots (areas of high microbial concentration and activity in comparison to the rest of the soil (Kuzyakov and Blagodatskaya, 2015). Furthermore, an effect of residue quality on crop quality was expected as it has already been shown in the previous experiment. Therefore, I hypothesized that, in field conditions, (i) the remaining residues in combination with crop residues at harvest, produce mulch of higher quality than that of the crop residues alone, (ii) residues of higher initial quality (low C:N ratio, high N, low recalcitrant substances) result in higher N mineralization than residues of lower quality at the later stages of decomposition, (iii) soil microbial biomass, at a later stage of decomposition, and crop quality (including crop morphology) are affected by residue diversity or quality.

Evolution of research ideas and thesis outline

The main interest in my research lies in the use of plant-based mulch of diverse mixtures of plant species to improve soil fertility and crop yield. The idea started preoccupying my thoughts during my first years of graduate studies as an agriculturist in the Aristotle University of Thessaloniki, back in 1986, where I had the opportunity to come across people and ideas relevant with agricultural practice and organic farming. One of the main principals in organic farming was that a healthy and fertile soil can grow healthy plants and provide high quality plant production (ORC, 2016). Therefore, the starting point of all my thoughts was how to improve soil fertility in the most sustainable way. Conventional agriculture, with its negative impacts on the environment and human health, had been identified as a potential problem already since 1950s, with the extensive use of artificial fertilizers, agrochemicals, and heavy machinery. The challenge to the green revolution began to emerge and culminated in the circulation of guides and regulations on organic farming since 1970s by worldwide organizations as the International Federation of Organic Agriculture Movements (IFOAM) or the Food and Agriculture Organization of the United Nations (FAO). Since my graduate studies, the use of compost, organic residues alone or mixed with soil, moistened, and biologically fermented (Brady and Weil, 2002) was considered by most farmers the most effective way to maintain soil fertility. However, it requires special effort, knowledge, space, and time, and very often access to ex-farm organic material to be produced. Therefore, it may not always be considered the most sustainable way of fertilization. Another established and well-tested method of fertilization is green manure produced by cover crops, incorporated into the soil to decompose before the next cash crop. Legumes can fix atmospheric N, thus enhancing soil N availability (Tribouillois *et al.*, 2015) and were traditionally used as green manure. However, green manure was usually incorporated into soil to rapidly decompose as it was thought to be more effective in providing soil nutrients than when applied as mulch. (However, green manure incorporation and even compost application require soil tillage which expose soil to erosion, water run-off, surface crusting, and destruction of soil aggregates, with severe negative effects on water infiltration, soil microbial activity, soil biodiversity, C sequestration, and nutrient availability (Carr *et al.*, 2013; Peterson *et al.*, 1998). Conversely, plant-based mulch was initially evaluated for its contribution to improve soil physical properties and to suppress weeds, but its effect on soil chemistry and biology has not been properly studied or appreciated. Moreover, mulching is a sustainable practice because it can be produced by farmers in their own fields, and easily applied with low cost. In recent decades, in parallel with the development of conservation tillage and no till practices, the value of biodiversity has become increasingly recognized in providing ecosystem stability and resilience. In this context, mulches of mixtures of plant species were increasingly tested whether they can provide a variety of ecosystem services instead of one plant species of green manure alone.

Soon after my graduation from University I started practicing and experimenting in mulching in an orchard for over 20 years where I had the opportunity to make interesting observations on the effectiveness of mulch on soil fertility and plant health. Residue diversity, biomass, and size, number

of mulch applications, and timing of application, heterogeneous distribution of residues on soil surface where all important factors requiring further investigation. The practical long-time experience, together with my academic background triggered my desire to seek answers to relevant research questions scientifically.

Based on this frame, I scheduled my first experiment in rhizotrons to investigate the effect of size (<1.5 cm vs 30 cm) and diversity of plant residue mulch on soil nutrient availability and soil microbes. According to bibliography and my observations as a farmer, shredded residues seem to be decomposed faster than long residues and logically provide higher amounts of nutrients at early stage of decomposition than long residues. However, long residues seem to cover and physically protect soil surface for longer period than short residues, but what about their direct contribution to soil fertility? Do they provide enough nutrients to support satisfactory crop yield in comparison to short residues? Do they provide more nutrients than short residues at later stages of decomposition? How do they influence crop seed quality in comparison to short residues? Do long residues maintain higher capacity as potential fertilizers than short residues at the end of the growing season? Does residue size affect beneficial symbiotic microorganisms like Arbuscular Mycorrhizal Fungi (AMF)? How does residue diversity (species richness and functional diversity) affect all these variables, and finally are long residues more appropriate to be used as mulch than short residues to support sustainable crop production? In order to answer these research questions I used residue material of diverse forages mixtures of 1, 6, 12, and 17 plant species from another ongoing long-lasting experiment, which I used during all my PhD research.

The results of my first experiment showed that indeed long residues maintain higher fertilizer capacity than short residues at the end of the growing season without deleterious effect on crop yield. Their contribution to enrichment of soil nutrients was not significantly different than that of short residues at later stages of decomposition. Moreover, there were indications that species richness could affect AMF root colonization.

Based on these results I scheduled the second rhizotron experiment using residue mulches of the same mixes of forage plants but only of long residues (30 cm) to evaluate the effect of diverse long-residue mulches on soil chemistry and microbiology, and on crop yield and crop quality, at later stages of decomposition. In this experiment I decided to add a treatment with wood chip residues consisted of shredded tree material as in many cases farmers, mainly in organic farming, use such material derived by pruning as mulch. In addition, wood chips were characterized by much lower quality (very high C:N ratio and recalcitrant substances) in comparison to the other treatments, and I was hoping it could reveal significant differences in the examined variables. In this experiment, although the same types of residue mixtures with the first experiment were used their quality characteristics were quite different between residues of the same type, because they were derived at different time periods. This

offers the opportunity to assess the effect of both species richness diversity and functional diversity of residues on the examined variables in comparison with corresponding results in the first experiment. Moreover, in the second experiment I decided to use Phospholipid Fatty Acid (PLFA) analysis to evaluate the effect of residue diversity on soil microbial biomass and on the fungal: bacterial ratio and interpret the results in comparison with results on other variables.

In parallel with experiment 2, I conducted a 3rd experiment in field conditions. In this field experiment using the same treatments with experiment 2 in order to evaluate the effect of the diverse long-residue mulches on soil chemistry and microbiology, and on crop quality, at later stages of decomposition in field conditions. In this experiment all plant residues except wood chips were consisted of aboveground whole plants, cut once on the soil surface. Again, the quality of each residue type was differing from the quality of the same residue type in the previous experiments, except wood chips. The remaining already largely decomposed plant residues at the end of the growing season were mixed with the crop residues after harvest, in order to evaluate whether the final quality of these mixed residues, and consequently their capacity as fertilizers for the next crop, were improved in comparison to the crop residues alone.

Research gaps

The contribution of plant-based mulch to the amelioration of soil physical conditions has been extensively investigated and appreciated. However, its capacity to improve soil fertility has been underestimated in favour of incorporated residues into soil, which are considered to have higher rates of decomposition than mulch. Moreover, most of the relevant research has been focused on the early stage of decomposition and concerns fine or coarse residue particle size of certain plant species incorporated into the soil. Especially in incubation experiments, where soil characteristics and climatic conditions are under control, incorporated shredded residues which are decomposed faster than long size residue mulch are preferably used because they are able to provide more distinctive differences between treatments (reduced error).

Most research looks at C and N mineralization or in the fate of P released from residues. Little attention has been given in K and Mg and other soil nutrients, although on certain occasions mulch has been found to solve micronutrient deficiency problems. Fine and incorporated residues are decomposed earlier than long residues in mulch, providing highly distinctive differences between treatments at the early stages of decomposition which is of interest to most researchers. Legumes, that are considered to enrich soil with N due to N fixation capacity, have been extensively used in relevant experiments. They are often claimed to be an indispensable constituent of cover crops to provide high-quality residues, alone or in bispecific mixtures with N catch-up species to avoid N leaching. However, the contribution of other plant species and families in soil fertility has been neglected or underestimated. There should be more research on mixtures of plant species as this is the usual case in field conditions.

The effect of mulch of plant species mixtures on microbial biomass, structure, and activity has not been thoroughly investigated. Residue diversity has been found to promote soil microbial diversity, but the subject needs further investigation, much more in terms of later stages of decomposition which may impact fertilization capacity of residues for the next cash crop. There is a gap in research on the effect of continuous application of mulch on soil fertility and soil microbial activity as well as on residue colonization by microorganisms, which is possible to affect the decomposition of a successive mulch application. In the same context, the fertilization capacity of the remaining residues and their final quality in combination with the crop residues, at the end of the growing season have not received due attention. Furthermore, special conditions existing in field experiments due to field variation and heterogeneous distribution of mulch worth thorough further investigation.

Research flow chart

Experiment 1 (Rhizotrons)

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Duration										
Plant emergence				26th						
Mulch application					3rd					
Planting					13th					
Sampling (d.a.m.a.)							70		137	

Experiment 2 (Rhizotrons)

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Duration										
Plant emergence				24th						
Mulch application				11th						
Planting					11th					
Sampling (d.a.m.a.)						69				195

Experiment 3 (field)

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Duration										
Plant emergence		8th								
Mulch application			31st							
Sampling (d.a.m.a.)				25	52	90	118			

d.a.m.a. - days after mulch application

Research gaps:

Experiment 1: 1) Long residues, 2) mulch of plant species mixture, 3) soil chemical properties (mineralization of soil nutrients other than N), 4) soil biological properties (abundance of AMF root colonization) 5) later stages of decomposition, 6) final residue fertilization capacity (at harvest).

Experiment 2: 1) mulch of plant species mixture, 2) soil chemical properties (mineralization of soil nutrients other than N), 3) soil biological properties (microbial biomass) 4) later stages of decomposition, 5) final residue fertilization capacity (long after harvest).

Experiment 3: 1) mulch of plant species mixture, 2) soil chemical properties in field conditions, 3) soil biological properties (microbial biomass) in field conditions 4) later stages of decomposition, 5) mulch quality of remaining residues in combination with crop residues (at harvest).

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Chapter 2

Paper 1

Fragment size and diversity of mulches affect their decomposition, nutrient dynamics, and soil microbiology

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Abstract

Plant-based mulch has been proposed as a sustainable way of maintaining soil fertility. However, the role of mulch diversity, quality, and size in decomposition dynamics, and their effect on crop yield are not fully explored. We investigated how mulch quality, proxied by the constituent plant species diversity, and residue size drive mulch decomposition, crop nutrition, and yield.

A rhizotron experiment was set up with barley as a model crop, with the addition of mulch of two particle sizes (1.5 and 30 cm) and four different plant residue mixes (17, 12, 6, and 1 species) in a fully factorial design. Soil nutrient dynamics were measured at advanced decomposition stages, together with residue quality, arbuscular mycorrhizal fungal (AMF) root colonization, and crop yield.

Residue mass loss was significantly affected by its chemical composition. *Long residues* retained significantly higher C and N content, than *short residues*. Crop yield was not affected by residue type or size. Residue size significantly affected barley growth rate, influencing seed protein content. Soil available K was significantly increased by residues with a higher initial C:N ratio. S*hort residues* resulted in higher soil Zn. Residues of higher diversity resulted in higher AMF root colonization of the barley plants.

Generally, l*ong residue* mulches maintain soil fertility for a longer period than *short* ones, without a deleterious effect on crop yield. Further investigation should evaluate the effect of continuous application of *long residue* mulches on soil fertility and microbial populations.

Key words: Plant-based mulch, residue size, residue diversity, residue quality, residue decomposition, soil nutrients.

Introduction

Plant-based mulch can significantly affect the physical, chemical, and biological properties of agricultural soils (Sharma et al., 2011; Mulumba & Lal, 2008; Abiven & Recous, 2007; Su et al., 2014). Mulches can provide physical protection from soil erosion and enhance and maintain soil fertility, particularly in conservation tillage, and organic farming systems (Peterson *et al.*, 1998; Blanco-Canqui et al., 2011; Carr et al., 2013). Sustainability in agroecosystems is typically defined by the reliance on local resources, and diversity conservation (Miglécz *et al.*, 2015). Therefore, mulch from cover crops and crop residues can contribute to sustainability through the promotion of soil fertility and the diversity of soil-dwelling organisms (Marinari et. al, 2015). However, in contrast to mineral fertilizers, the release of nitrogen from organic sources sometimes is unpredictable and much more susceptible to variations in environmental conditions (Rodrigues *et al.*, 2013). The effect of mulching on soil carbon (C) and nitrogen (N) – as indicators of soil quality - is not well understood (Calderón et al., 2016). Generally, the greater the mass of residues, the higher the soil C content, yet the decomposition curve of N is not so straightforward and is affected by a number of soil conditions (Dietrich *et al.*, 2017).

The timing of nutrient release relative to crop demand through the growing season has been found to be an important issue when using mulch. Su et al. (2014) found increased N uptake and growth by plants during the early stages of their development, soon after mulching application. They note that these effects were diminishing with time, probably due to enhanced ammonia volatilization. However, soil N, mainly NH_4^+ and organic N availability, does not always increase immediately after mulch application. Siczek et al. (2011) showed that N availability after mulch application could be limited during crop vegetative stages when soil nitrate content is low. Moreover, a rapid decomposition is not always beneficial, as it may provide nutrients too early, leading to a lack at latter stages (Marinari *et al.*, 2015).

Concentrations of available nutrients can also be dependent on the number of applications of plant mulch. Pavlu et al. (2016) observed increasing concentrations of available P and K in ascending order from one to three annual applications. P concentrations are typically higher in topsoil due to the decomposition of organic materials and plant residues, to rapid fixation by soil particles, and to immobilization by microorganisms (Lynch and Brown, 2001). Moreover, higher seed protein content in soybean was reported with mulch application, whereas there were no measurable effects on yield in comparison to no mulch (Siczek and Lipiec, 2011).

Residue diversity can stimulate soil ecosystem services but there is a paucity of knowledge on the type (plant traits) and the quantity (species richness) of the diversity required (Finney et al., 2016; White et al., 2017). When plant residues are derived from a mixture of plant species, their decomposition process can be either faster (synergistic effect), slower (antagonistic effect), or proportional to constituents (additive), depending on the individual plants which participate (Redin et al., 2014). Crop residues of a mixture of different plant species may increase plant residue-derived C assimilation by

soil microbes in the early stage of decomposition, than residues of single plant species, due to functional complementarity which results in reduced competition between microbial communities (Shu *et al.*, 2022). Substrates of different chemical composition are decomposed by different groups of microbes, using different enzymes (Fontaine et al., 2003). Therefore, residues with high plant diversity favour microbial diversity (Wu et al., 1993) and arbuscular mycorrhizal fungal (AMF) diversity (Burrows and Pfleger, 2002).

The decomposition of plant residues can be affected by the amount of contact between soil particles and plant material and consequently by the size of plant residue per unit mass. Shredding mulch residues to small particle size breaks up the continuity of recalcitrant plant tissues, potentially aiding decomposition. This makes the mulch easier to decompose by increasing accessibility and surface area available to soil microbes (Angers & Recous, 1997). Residue size interacts with the C:N ratio and the availability of N in the soil. For example, the decomposition of *short* vs *long residues* may not be accelerated, despite higher contact with soil, when residues are rich in N (Bremer et al., 1991). The particle size of straw residues (high C:N ratio) did not play an essential role in N immobilization and soil microbial biomass in field conditions with low soil inorganic available N (Jensen and Ambus, 1994). The immobilization, mineralization, and denitrification of N were higher in shredded residues with high C:N ratio in N rich soil, but only in the short term (Bremer et al., 1991; Angers and Recous, 1997). This suggests that residue particle size does not affect the final amount of nitrogen that is released in the soil but only the timing of the release and availability to plants.

This study aims to improve our understanding of the optimal size of mulch fragments (whole plant versus shredded) and the importance of plant residue diversity. We hypothesise that (i) *long residues* maintain higher fertilization capacity than *short residues* at the end of the growing season, (ii) soil nutrient availability at the later stages of decomposition are affected by both residue size and residue quality (residue C:N ratio, N, and recalcitrant substances), (iii) AMF root colonization increases with increasing residue species richness, (iv) and crop quality is affected by both residue size and residue quality but with no deleterious effect on crop yield.

Our results are discussed in the context of long-term soil health and fertility, with the view of informing the optimisation of long-term mulch use.

Methodology

Rhizotron setup

The experiment was conducted at the Crop and Environment Laboratory of the University of Reading in the UK, from June to November 2018. Thirty-six minirhizotrons were constructed from 0.5 cm thick PVC sheeting, each representing an independent replicate (Fig. 1). One side of each rhizotron was clear acrylic to allow observation of root growth and was removable to allow soil sampling. Between

observations and sampling, the clear acrylic was covered with thermawrap silver foil to avoid light penetration and to minimize temperature fluctuation. Each rhizotron was 1 m tall to allow root system expansion at depth, and 30 x 5 cm wide, thus providing soil volume of 0.015 m^3. A layer of gravel c.1.5 cm thick was placed at the bottom to allow drainage, the rest was filled with c.20kg of commercially supplied well-homogenized loamy sand topsoil (bulk soil) with pH 7.3 ± 0,032 SD and sieved to 8 mm. The rhizotrons were kept at 70^o angle for the duration of the experiment, with the transparent side facing down so that the roots grow along the transparent side for better observation. Initially, they were placed outside and moved into a greenhouse in October to aid plant senescence.

Experimental design

Spring barley (*Hordeum vulgare L., var. Laureate*) seeds (56 mg to 66 mg weight) were sown on 11th June 2018 into a germination tray. Plant emergence occurred after 15 days, vigorous plants between 15 cm and 22 cm height were then transplanted into rhizotrons 17 days later. Two plants per rhizotron were planted 15 cm apart and 7.5 cm from each edge (Fig. S1). An automated irrigation system with four drippers per rhizotron was installed to maintain moisture at 55-60% Water Holding Capacity (WHC). Irrigation stopped 106 days after plant emergence to promote seed maturity. Growth stages of the barley plant were observed every 1 to 2 weeks, and the plants were harvested 121 days after mulch application.

Eight biomass residue treatments were established in a fully factorial design (n=4), comprising four plant diversity mixtures (types) and two plant residue particle sizes (*short* of 1.5 cm *(S),* and *long* of 30cm *(L)* so long as the length of the soil surface in a rhizotron). A control *(C)* with no mulch application was also established. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass™ ***(S)*** (6 species), Biomix™ ***(B)*** (12 species), and Herbal™ ***(H)*** (17 species) (Table S1), provided by another project (the DiverseForages Project). These residue types were suitable to address our hypothesis because they included residue mixtures with different characteristics. Residue characteristics include both residue diversity and functional traits. Residue diversity may concern either the species composition or the species richness which is the number of species participating in a residue mixture. The functional traits of residues may concern either the chemical composition, which determines the residue quality, or the morphological features of residues (Santonja *et al.*, 2018; Hättenschwiler et al., 2011). Fresh plant residue was collected from field plots planted with the aforementioned forage mixtures on 29th June 2018, cut to specified sizes, and placed on the soil surface in rhizotrons randomly allocated to each treatment 4 days later. Plant mass representative of 23g dry mass was used in each treatment. Thus, the 9 treatments were: ***HL, HS, BL, BS, SL, SS, PL, PS, and C.*** No additional fertilization or pesticide treatment was applied to the rhizotrons during the experiment. The encoding $P_{(1)}$, $S_{(6)}$, $B_{(12)}$, and $H_{(17)}$ as well as $PL_{(1)}$, $PS_{(1)}$ $SL_{(6)}$, $SS_{(6)}$, $BL_{(12)}$, $BS_{(12)}$, $HL_{(17)}$, and $HS_{(17)}$ has been adopted where it was

considered necessary to indicate the number of species participating in the different residue types or treatments, respectively.

Soil and plant material sampling and analyses

Soil samples were taken from rhizotrons on two occasions: 70 and 137 days after mulch application (Fig. 1). In total, 21 soil samples were collected from each rhizotron (4 in the first sampling period and 17 in the second), making a total of 756 from all rhizotrons (Fig. S2). All samples, except those with roots, were air-dried and then sieved to <2mm and stored at room temperature. Soil samples with roots were stored at 4°C until root extraction. Roots were extracted by submerging soil samples in tap water over a 1 mm sieve and collecting all floating roots.

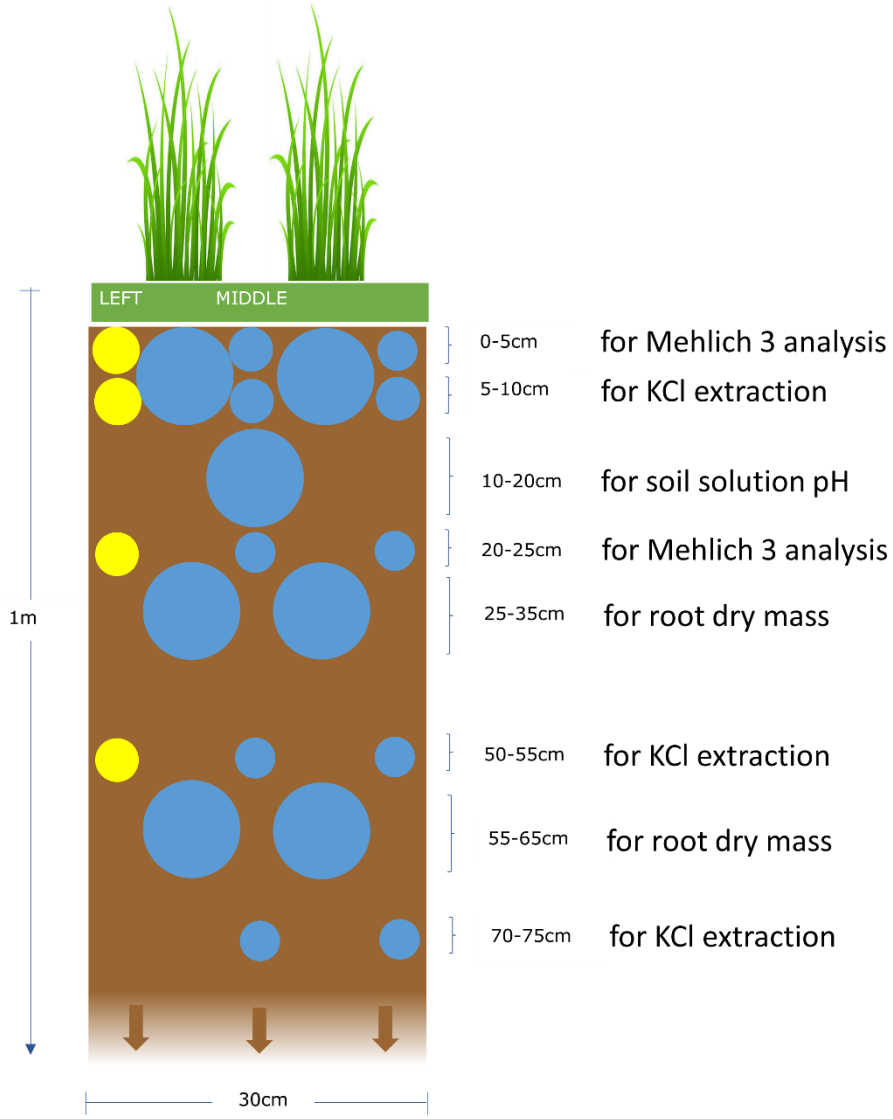

Fig. 1 Rhizotron and soil sampling design. Large circles indicate root extraction samples, except for the 10-20 cm depth which was used to measure soil solution pH and bulk density. The large circles at 0-10 cm depth were used to detect AMF colonization in barley roots. Yellow highlighted samples were taken on day 70, blue highlighted on day 137 after mulch application

After the final harvest, mulch residue, aboveground and belowground barley plant biomass, and soil samples were analyzed to establish key physical and chemical properties of investigated plant-soil systems. Mulch residue and barley biomass were dried for 48 to 72 hours at 80^{o}C until constant weight and then at 105^{o}C for 24 hours to establish dry weight. Total carbon, nitrogen, and protein content of mulch, plant tissue, and seeds, together with total carbon and nitrogen of soil sub-samples, were determined through combustion (LECO CHN 628 analyser, LECO Corporation). ANKON 200 Fibre Analyser (ANKON Technology) was used to measure percentage content of neutral detergent fibre (NDF) (Hemicellulose, Cellulose, and Lignin), acid detergent fibre (ADF) (Cellulose and Lignin), and acid detergent lignin (ADL) (Lignin) of mulch residues according to ANKOM Technology protocols. We then estimated the % Cellulose by subtracting the % ADL from % ADF, and the % Hemicellulose by subtracting the % ADF from the% NDF. Samples were first dried at 80^{o}C to constant weight and ground in a Fritsch grinder (Glen Creston Ltd) with 1mm sieve. Arbuscular mycorrhizal fungi (AMF) colonization and abundance was estimated by black ink staining according to Vierheilig et al. (1998).

Air-dried 10 g soil samples sieved to <2 mm were suspended in centrifuge tubes in 25 mL of ultra-pure water and shaken for 15 minutes on an end-over-end shaker to measure soil solution pH (Blakemore et al., 1987). Available N was estimated by KCl extraction method according to standard protocol (Great Britain. M.A.F.F., 1986) using 40 g of air-dry soil in 200 ml 1M potassium chloride solution, measured colorimetrically by a San Continuous Flow Injection Analyzer (SCALAR Instruments). The Mehlich 3 method (Mehlich, 1984) was used to evaluate the availability of P, K, Mg, Mn, Fe, Cu and Zn nutrients by Perkin Elmer – Optima 7300 DV ICP-OES analyser (Pierzynski, 2000; AgroEcoLab UMD; PerkinElmer, Inc.). Air dry soil samples of 2 g and 20 ml of Mehlich 3 extracting solution were used. Other soil samples were air-dried and sieved to <2mm to establish soil texture, which was measured with a hydrometer by adding 50 ml sodium hexametaphosphate solution (SHMP) at 50 g/l to 40 g soil subsamples (Bouyoucos, 1962). Soil water holding capacity (WHC) was estimated gravimetrically using 50 g fresh soil samples with the method described by Harding and Ross (1964). Three samples were taken from the middle of three rhizotrons in random from the depth of 12-16 cm to measure average bulk density (Gradwell and Birrell, 1979). The height of the main stem of barley plants from the soil surface to the base of the flag leaf was used as a proxy of growth rate (cm/d).

Statistical analysis: All statistical analyses were carried out in Minitab 19 (Minitab, LLC) except Principal Component Analysis which was conducted in R-Studio (RStudio, PBC). All measurements from a rhizotron were averaged to obtain a single mean value, the unit of replication of this study being the rhizotron (n=4), and $\alpha < 0.05$ was used to denote significance. One-way ANOVA and a General Linear Model were used to detect differences between treatments in the case of one factor (treatments, residue type, or residue size) and two factors (type and size of residues), respectively. Measurements repeated in time (day 70, and day 137) or position (middle and right side of rhizotrons) or soil depth were analyzed by Mixed Effects Model with rhizotrons as random factor and type of residues, size of

residues, time, depth, and position as fixed factors. All data subjected to analyses of variance were tested for normality and homogeneity, using Darling-Anderson and Levene tests, respectively. When these conditions were not satisfied, data were transformed by $\log_{10}$ or Box-Cox transformation with optimal or rounded λ. Kruskal-Wallis test was conducted instead of one-way ANOVA if transformations did not normalize data variance sufficiently. When a significant treatment effect was observed, a Tukey post-hoc test was also conducted. Comparisons with the control treatment were made using the Dunnett test. Data were separated and analyzed with ANOVA for a specific sampling time or depth, when necessary, where normality or equality of variances were not satisfied even after data transformation. Descriptive statistics included means and standard deviations rather than standard errors unless it was otherwise stated.

Simple regression analysis was conducted in order to examine the significance and the degree of the effect of a single independent variable to a response variable in cases where there was an apparent influence (residue dry mass loss vs initial residue NDF or N content or initial residue C:N ratio, soil K vs initial residue C:N ratio, AMF root colonization vs residue species richness, seed protein vs main stem elongation rate as continuous predictor variable and residue size as categorical predictor variable). Homogeneity of variance of the data was tested with Levene test, and normality of data distribution with Anderson-Darling test. The Spearman correlation was used when normality or equality of variances of data were not satisfied. Pearson correlation was used to assess the significant correlations between variables.

Multivariate analysis was also conducted on data collected from barley plants and soils using Principal Component Analyses (PCAs) to assess differences between treatments in several variables simultaneously, concerning measurements on barley plants or soil nutrients, and the relations of the treatments with those variables.

Results

Residue dry mass loss, and initial and final quality

Residue (mulch) dry mass loss due to decomposition was significantly different between treatments (N = 4, F = 2.57, p-value = 0.040) and specifically between $BS_{(12)}$ (71.71 ±7.21) and $PL_{(1)}$ (47.78 ±6.44) (T-value = -3.40, p-value = 0.041, Fig. 2).

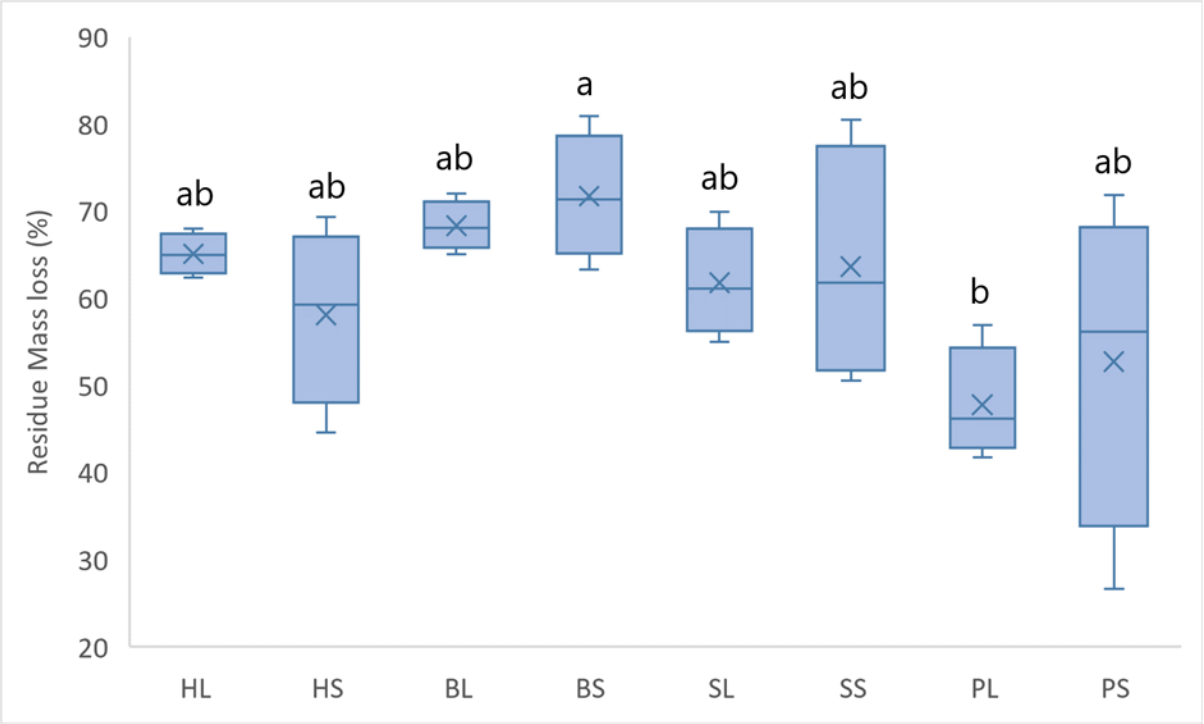

Fig. 2 Final residue dry mass loss (121 days after mulch application). Mean values are depicted with x (N = 4, F = 2.57, p-value = 0.040). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (*short* of 1.5 cm *(S),* and *long* of 30cm *(L)*). Treatments that do not share a common letter are significantly different ($p < 0.05$)

Further analysis showed a significant effect of residue diversity (residue types) on residue dry mass loss (N = 8, F = 5.40, p-value = 0.006). There were significant differences between $P_{(1)}$ (50.26 ±13.33) and $B_{(12)}$ (70.02 ±5.39) (T-value = -3.98, adjusted p-value = 0.003), but no significant between *long* and *short* residue size (N = 16, F = 0.05, p-value = 0.823).

The $P_{(1)}$ residue type had the lowest initial C:N ratio (24.2 ±2.268) while $S_{(6)}$ had the highest (33.5 ±1.65), $B_{(12)}$ had 30.4 ±3.64 and $H_{(17)}$ 28.1 ±1.801 (N = 8, F = 20.24, p-value < 0.001, Tables S2 and S3). Type $P_{(1)}$ had the highest mean value (41.644 ±0.160) of initial C content of residues, significantly different from $B_{(12)}$ (41.229 ±0.256), and $H_{(17)}$ (41.207 ±0.195) (N = 8, p-value = 0.001, Tables S2, and S3). $S_{(6)}$ type had the lowest initial N content (1.238 ±0.055) while $P_{(1)}$ had the highest (1.734 ±0.170), and there were significant differences between types (N = 8, F = 19.55, p-value < 0.001, tables S2 and S3). The same pattern was observed in initial protein content of residues (N = 8, F = 19.55, p-value < 0.001). There were no significant differences in initial ADL (lignin) content between residue types (N = 4, Kruskal-Wallis test p-value = 0.277, Fig. S3, Table S4). In addition both NDF (hemicellulose + cellulose + lignin) and ADF (cellulose + lignin) contents include lignin. There was a significant positive correlation between NDF and ADF (r = 0.938, p-value < 0.001) and no significant correlation between NDF and lignin (r = -0.021, p-value = 0.937) or between ADF and lignin (r = -0.058, p-value = 0.832). Moreover, differences between residue types in ADF were statistically more significant (N = 4, F = 5.76, p-value = 0.011) than

in cellulose alone (Kruskal Wallis test, N = 4, H-value = 6.60, p-value = 0.086), while it was of equal significance in both NDF (N = 4, F = 16.14, p-value < 0.001) and in hemicellulose alone (N = 4, F = 23.04, p-value = < 0.001) (Fig. S3, Tables S4 and S5). For these reasons, NDF was used in the experiment as a measure of residue recalcitrance.

Residues harvested at the end of the experiment showed significant differences in C:N ratio between treatments (N = 4, F = 4.27, p-value = 0.003). $PL_{(1)}$ treatment which had the highest mean value was significantly higher than $HS_{(17)}$ and $BS_{(12)}$ (Fig. 3a, Tables S6 and S7). Further analysis showed significant differences in final C:N ratio between types (N = 8, F = 7.57, p-value = 0.001) as well as between *short* (15.029 ±2.099) and *long* (16.744 ±2.595) residues (N = 16, F = 6.66, p-value = 0.016). The C:N ratio was significantly higher in $P_{(1)}$ (17.959 ±2.407) than in $B_{(12)}$ type (13.883 ±1.797) (T-value = 4.33, adjusted p-value = 0.001), in $P_{(1)}$ than in $H_{(17)}$ type (14.922 ±2.000) (T-value = 3.23, adjusted p-value = 0.018), and in $S_{(6)}$ (16.786 ±1.655) than in $B_{(12)}$ type (T-value = 3.09, adjusted p-value = 0.024). Final C content of residues was also significantly higher (59.20%) in *long* residues (30.95 ±4.68) than *short* residues (19.44 ±5.89) (N = 16, F = 36.74, p-value < 0.001). $PL_{(1)}$, $SL_{(6)}$, and $HL_{(17)}$ were significantly higher than $BS_{(12)}$, $HS_{(17)}$, and $PS_{(1)}$ (N = 4, F = 6.02, p-value < 0.001, Fig. 3b, Tables S6 and S7). There were significantly higher final N contents in $HL_{(17)}$, $BL_{(12)}$, $SL_{(6)}$ treatments than in $HS_{(17)}$, and $PS_{(12)}$ (N = 4, F = 5.68, p-value = 0.001, Fig. 3c, Tables S6 and S7). In addition, final N content of residues was significantly higher (44.06%) in *long residues* (1.854 ±0.154) than *short* ones (1.287 ±0.303) (N = 16, F = 38.05, p-value < 0.001).

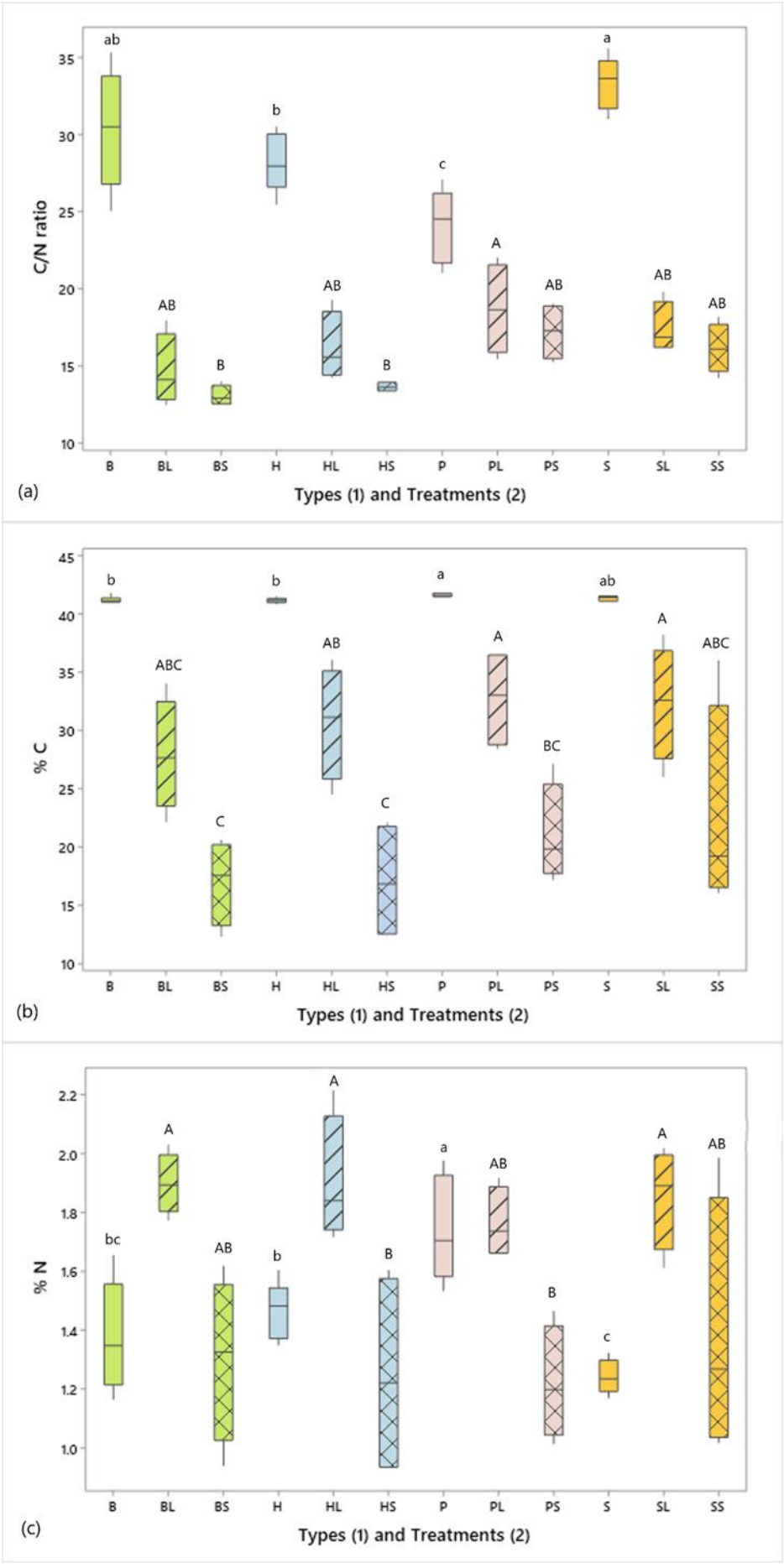
C/N ratio
% C
% N
Types (1) and Treatments (2)
B
BL
BS
H
HL
HS
P
PL
PS
S
SL
SS
(a)
(b)
(c)

Fig. 3 Box and whiskers plots of plant residue initial and final (a) C:N, (b) % C, and (c) % N ratio for the different types (*B, H, P, and S*) (initial residues) and Treatments (*BL, BS, HL, HS, PL, PS, SL, and SS*) (final residues). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (*short* of 1.5 cm *(S),* and *long* of 30cm *(L)*). Lower case letters refer to comparisons between initial residue types. Upper case letters refer to comparisons between final residue treatments. Mean values that do not share a common letter are significantly different ($p < 0.05$)

Table 1 Classification of residue types (*H, B, S, and P*) and treatments (*HL, HS, BL, BS, SL, SS, PL, PS*, and *C*) in descending order of their initial or final mean values according to different properties. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (*short* of 1.5 cm *(S),* and *long* of 30cm *(L)*). Control treatment (*C*) was with no residues. NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin)

Properties	**Residue types**	**Properties**	**Treatments**
Initial C:N ratio	$S^{a} > B^{ab} > H^{b} > P^{c}$	Final C:N ratio	$PL^{a} > SL^{ab} > PS^{ab} > HL^{ab} > SS^{ab} > BL^{ab} > HS^{b} > BS^{b}$
Initial % C	$P^{a} > S^{ab} > B^{b} > H^{b}$	Final % C	$PL^{a} > SL^{a} > HL^{ab} > BL^{abc} > SS^{abc} > PS^{bc} > HS^{c} > BS^{c}$
Initial % N	$P^{a} > H^{b} > B^{bc} > S^{c}$	Final % N	$HL^{a} > BL^{a} > SL^{a} > PL^{ab} > SS^{ab} > BS^{ab} > HS^{b} > PS^{b}$
% initial NDF	$P^{a} > S^{b} > B^{bc} > H^{c}$		
% initial ADF	$P^{a} > S^{ab} > B^{b} > H^{b}$		
% initial ADL	$S > H > P > B$		
Diversity	$H > B > S > P$		

Types or treatments of the same property that do not share a common letter are significantly different ($p < 0.05$).

There were no statistically significant correlations between C and N content in final *long* residues: $HL_{(17)}$ r = 0.518 (p = 0.482), $BL_{(12)}$ r = 0.461 (p = 0.539), $SL_{(6)}$ r = 0.801 (p = 0.199), $PL_{(1)}$ r = -0.199 (p = 0.801). In contrast, there were significant positive correlations between C and N content in final *short* residues in all treatments except in $PS_{(1)}$ treatment: $HS_{(17)}$ r = 0.999 (p = 0.001), $BS_{(12)}$ r = 0.970 (p = 0.030), $SS_{(6)}$ r = 0.970 (p = 0.030), and $PS_{(1)}$ r = 0.869 (p = 0.131). The same was true considering all *long* residue (r = 0.260, p = 0.331) and all *short* residue (r = 0.875, p < 0.001) treatments together.

Simple linear regression analysis was conducted using the residue dry mass loss as response variable (y) and either the initial NDF content or the initial N content or the initial C:N ratio of residues (x) as predictor variable (N = 8). The analysis showed that the regression model could explain only r^2 = 21.12% or 24.50% or 21.36%, respectively of the variation of the response variable. However, the influence of the predictor was statistically significant in all cases (p-value = 0.008 for NDF, 0.004 for N, and 0.008

for C:N ratio). The regression equations were y = 113.1 - 1.190x for NDF, y = 106.2 - 30.99x for N, and y = 16.2 + 1.548x for C:N ratio.

Soil nutrient content

Soil samples taken at harvest time on day 137 from 10-20 cm depth from the middle of the rhizotrons showed no significant differences in soil solution pH between treatments (N = 4, F = 0.40, p-value = 0.909).

The effect of residue diversity and of residue size on soil NO_3^- content was not significant on day 70 at 50-55cm depth (the only timepoint and depth that they were detected), (N = 4, F = 0.46 and p-value = 0.712 for type, F = 0.30 and p-value = 0.591 for size). However, mean value of all treatments (1.407 ±0.852) was significantly lower than that of bulk soil (initial soil, prior to its use in rhizotrons) (11.334 ±1.533, N = 4, F = 40.80, p-value < 0.001).

Soil NH_4^+ content was highly significantly lower on day 70 (1.302 ±0.356) than day 137 (1.678 ±0.379) (N = 64, F = 46.65, p-value < 0.001) and significantly higher at the top 5-10 cm (1.689 ±0.280) than at 50-55 cm (1.292 ±0.428) depth (N = 64, F = 52.13, p-value < 0.001, Tables S8, S9, and Fig. S4).

Depth significantly affected all soil nutrient contents (P, K, Mg, Fe, Mn, Zn, and Cu), and time significantly affected all nutrients except Fe and Mn (Tables S10 and S11). Macronutrients P and Mg had higher mean values at 20-25 cm depth than at 0-5 cm, but the opposite was true for K. Macronutrient concentrations were higher on day 70 than on day 137 at both 0-5 and 20-25 cm depths. Further analysis showed significant differences in soil K content between diverse residue types on day 137 at 0-5 cm depth (N = 8, F = 4.16, p-value = 0.017, Fig. 4). Tukey's post-hoc testing showed significant differences between $S_{(6)}$ (mean = 102.60 ±31.70) and $P_{(1)}$ (68.66 ±13.11) (T-value = 2.92, p-value = 0.035), and between $S_{(6)}$ and $B_{(12)}$ (68.44 ±24.12) (T-value = 3.17, p-value = 0.020) types. Spearman's correlation confirmed there was a positive and statistically significant association between the soil K content and the initial C:N ratio of residues (Spearman ρ = 0.439, p = 0.007).

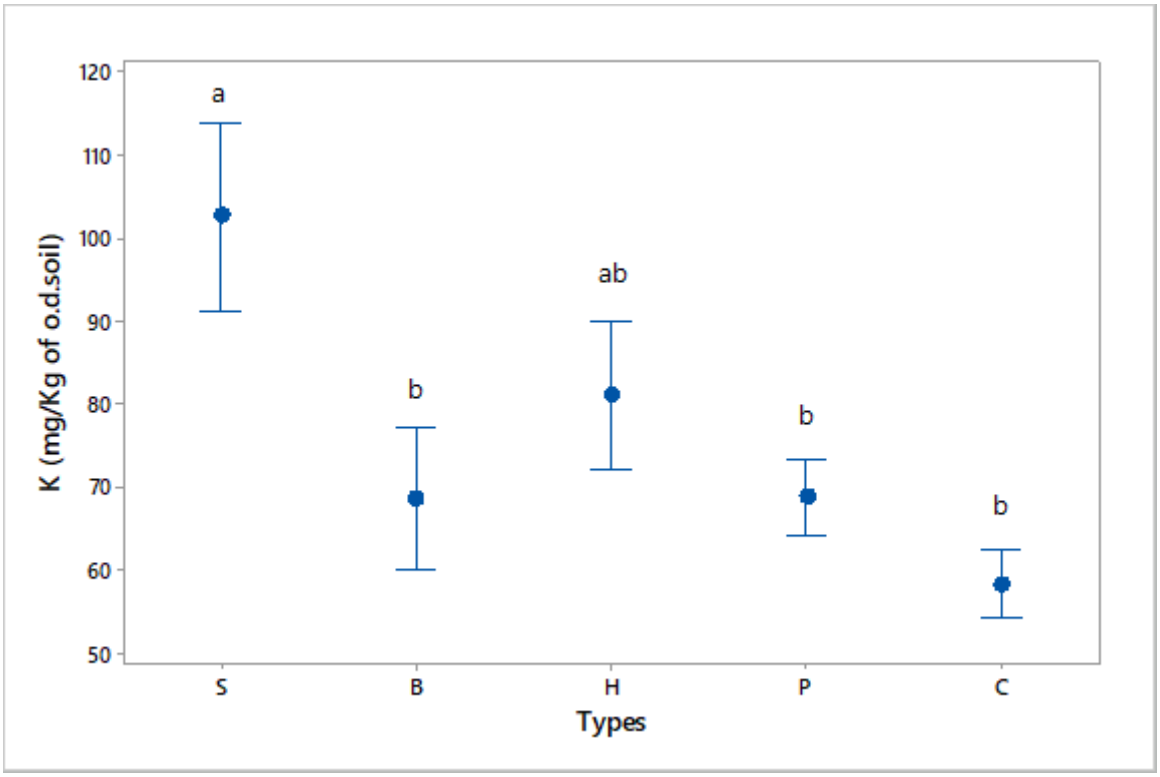

Fig. 4 The effect of different types (*S, B, H, P* in descending order of their initial C:N ratio) of residues on soil K concentration (Mehlich 3 extraction), in comparison to unamended Control *(C)*, 137 days after mulch application. Results include all types, and depth of 0-5 cm (N = 8). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Means and bars of one standard error from the mean are depicted. Types that do not share a common letter are significantly different (p-value < 0.05)

All micronutrients (Fe, Mn, Zn, and Cu) had significantly higher mean values (p-values < 0.001) at 0-5 cm depth than at 20-25 cm. Further analysis revealed significantly lower soil Zn content (-38.30%) in *long* residues (21.73 ±8.77) than *short* (35.22 ±15.69) on day 137 at 0-5 cm depth (N = 16, F = 7.53, p-value = 0.011).

The influence of the different treatments on soil nutrients (P, K, Mg, Fe, Mn, Zn, and Cu) on a multivariate basis, at the depths of 0-5 cm and 20-25 cm, was tested in Principal Component Analyses on day 70 (Fig. S5), and on day 137 after mulch application (Fig. S6). Although in most cases treatments were overlapping, *Control* seemed to demonstrate the most negative relation with soil nutrients in comparison to the other treatments, most clearly at 0-5 cm depth on day 70 (Fig. S5a). Also the contribution of $S_{(6)}$ type in enrichment of soil K in comparison to the other treatments was obvious at 0-5 cm depth on day 137 (Fig. S6a).

Cultivation of barley resulted in statistically significant reduction in soil K (N = 4, Kruskal-Wallis p-value < 0.001) and Mn (N = 4, F = 2.50, p-value = 0.011) in comparison to the bulk soil.

Samples taken from the middle of the rhizotrons, the area of plant root interaction, on day 137 revealed significant differences in soil nutrient contents in comparison to samples from the right side of rhizotrons, considering both samples from 0-5 and 20-25 cm depths (N = 64, F = 16.27, p-value < 0.001 for soil K, F = 56.91, p-value < 0.001 for P, F = 6.60, p-value = 0.012 for soil Mn, F = 14.52, p-value < 0.001 for soil Zn, and F = 16.51, p-value < 0.001 for soil Cu). Therefore, the effect of plant root interaction should be considered in experiments.

Barley plants

Residue size significantly affected the length of ears (N = 32, F = 4.96 and p-value = 0.030). Treatments with *short* size residues had higher mean values (8.337 ±1.453) than those with *long* size (7.778 ± 0.052). Total yield was not significantly affected by the different treatments (N = 8, F = 0.29, p-value = 0.968). Likewise, barley seed protein content was not statistically significantly different between treatments of long and short residues (N = 4, F = 1.90, p-value = 0.182) or between treatments of different types (N = 4, F = 1.50, p-value = 0.242). However, in all types except $P_{(1)}$ all treatments with long size residues had higher values of protein content than treatments of the same type with short size residues, and Control had lower value than any treatment with long residues (Table S12). Spearman correlation showed a significant negative correlation (Spearman ρ = -0.529, p-value = 0.001) between length of ear and seed protein content (%).

The Arbuscular Mycorrhizal Fungi (AMF) root colonization, detected on day 137, showed no significant differences as a result of treatments (*HL, HS, BL, BS, SL, SS, PL, PS, C*) (N = 4, F = 1.16, p-value = 0.356) or residue size (N = 16, F = 0.09, p-value = 0.772). However, mean values indicated that residues of higher species richness had higher AMF colonization. *Control* had the lowest mean value (Table 2).

Table 2 Mean values and standard error of AMF root colonization (%) in all treatments (*HL, HS, BL, BS, SL, SS, PL, PS,* and *C*) at harvest time (on day 137 after mulch application). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (*short* of 1.5 cm *(S),* and *long* of 30cm *(L)*). Control treatment (*C*) was with no residues. Mean values and standard errors are included (N = 4)

Treatments	**Mean ±SE**	**Treatments**	**Mean ±SE**	**Treatments**	**Mean ±SE**
HL	**35.52 ±1.67**	**SL**	**37.94 ±3.25**	**C**	**28.84 ±2.34**
HS	36.27 ±4.30	*SS*	32.42 ±2.70		
BL	**34.88 ±3.44**	**PL**	**29.35 ±1.54**		
BS	35.41 ±2.33	*PS*	31.05 ±3.99		

Linear regression analysis showed that residue species richness could explain only r^2 = 15% of the AMF root colonization variation. However, the influence of the predictor was statistically significant (N = 8, p-value = 0.02). The regression equation was y = 30.60 + 0.365x, which means that for every plant species that is added in the residue mixture an increase of 0.365% in AMF root colonization was expected (Fig. S7).

Barley plant growth rate

All treatments followed the same pattern of stem elongation (Fig. 5).

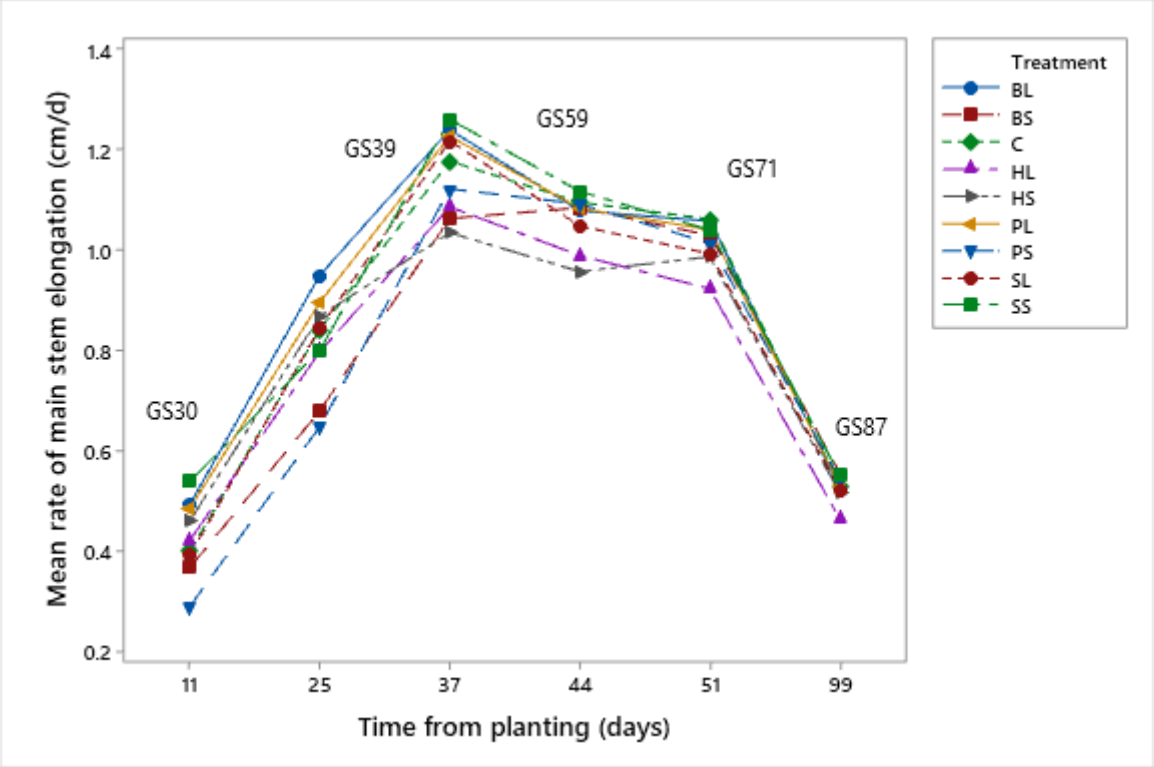

Fig. 5 Mean rate of main stem elongation (cm/d) of barley plants from soil surface to the base of the flag leaf for the different treatments (*BL, BS, C, HL, HS, PL, PS, SL, SS,* and *C*), 11, 25, 37, 44, 51, and 99 days after planting (28, 42, 54, 61, 68, and 116 days after plant emergence). GS = growing stage (Tottman et al., 1986). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (*short* of 1.5 cm *(S),* and *long* of 30cm *(L)*). Control treatment *(C)* was with no residues

Main stem elongation rate (cm/d) was significantly higher in *long* residues (0.871 ±0.230) than *short* residues (0.749 ±0.257) 25 days after planting (42 days after plant emergence) (N = 16, F = 4.00, p-value = 0.050). However, 99 days after planting (116 days after plant emergence) main stem elongation in *long* residue treatments were significantly lower (0.509 ±0.061) than in *short* ones (0.540 ±0.0521) (N = 16, F = 4.98, p-value = 0.003). Contrary, residue type did not affect significantly main stem elongation rate.

Linear regression analysis of seed protein content (%) as response variable, main stem elongation rate (MSER) as continuous predictor variable, and residue size (*long, short*, and *Control*) as categorical predictor variable on day 25 after planting was conducted (Fig. S8). The analysis showed that main stem elongation rate could explain only r^2 = 15.13% of the final seed protein content, but the influence was significant (N = 8, F = 4.62, p-value = 0.040). The regression equations were:

Protein = 6.453 + 0.644 MSER for *Control*,

Protein = 6.577 + 0.644 MSER for *long residues*, and

Protein = 6.596 + 0.644 MSER for *short residues*.

The influence of the different treatments on several variables concerning barley plant biomass (variables v1 and v2), the relation of barley plants with symbiotic microbes (variable v3), and seed quality and yield (variables v4 to v6) on a multivariate basis, was tested in a Principal Component Analysis (Fig. 6).

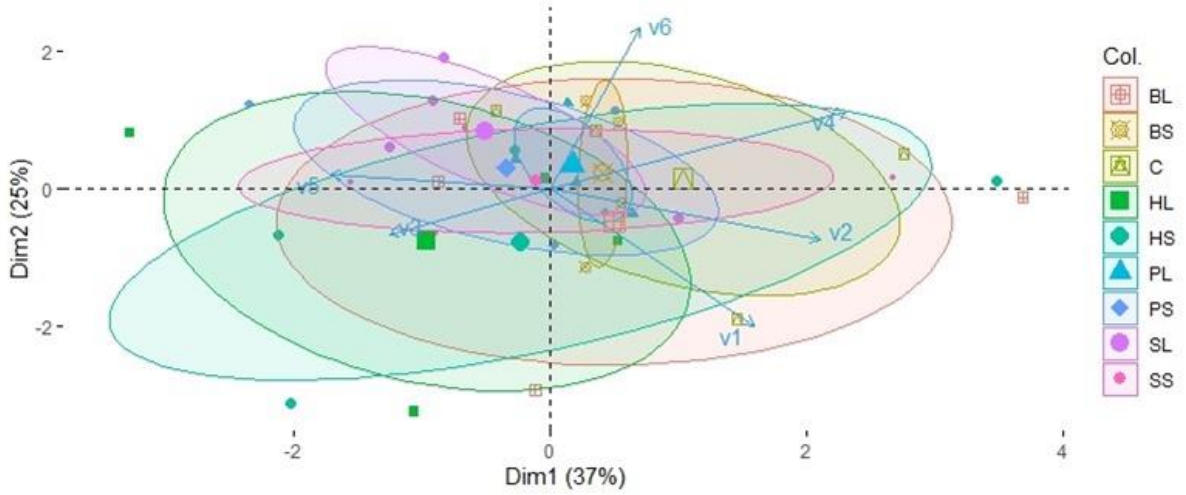

Fig. 6 PCA ordination of variables v1 = Barley root dry mass (g) (from combined samples at 25-35cm and 55-65 cm depth), v2 = barley plant dry mass (g) per plant per rhizotron (without ears and roots), v3 = % AMF colonization in barley roots, v4 = total seed dry mass (g) per plant per rhizotron, v5 = % barley seed protein content, v6 = % barley seed carbon content, for the treatments *HL, HS, BL, BS, SL, SS, PL, PS,* and *Control* (*C*) concerning measurements on barley plants. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (*short* of 1.5 cm *(S),* and *long* of 30cm *(L)*). Control treatment (*C*) was with no residues. Large points depict mean values, while ellipses depict confidence intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1 normalized score, and left axis represents Dim2 normalized score

In Fig. 6 it is evident, $HL_{(17)}$ treatment had the strongest positive relationship with AMF colonization (v3 variable) and the strongest negative with total seed dry mass (v4), opposite *Control* treatment. In the rest of variables differences between treatments were not clear confirming the lack of significant differences as treatments were highly overlapped.

Discussion

Residue quality and degree of decomposition

The initial C:N ratios of the residue types were all significantly different, ranging between 15 and 33. Soil decomposer microbes have a C:N ratio around 8 in their body and assimilate about 1/3 of the decomposed C. Therefore, an initial residue C:N ratio ≤24 is considered to drive to net N mineralization even at later stages of decomposition contrary to residues with C:N ratio >24 (Trinsoutrot *et al.*, 2000). However, this threshold may be risen to more than 30 by the activity of soil fauna, but normally <35 (Frouz *et al.*, 2015; Brust, 2019). C:N ratio affects organic matter decomposition in N-poor soils, while its effect in N-rich soils can be insignificant (Bonanomi et al., 2017). Apart from C:N ratio, decomposition is greatly affected by residue recalcitrance as well (Vahdat *et al.*, 2011). Interestingly, $P_{(1)}$ type had the lowest C:N ratio but the highest recalcitrance, not significantly different from that of $S_{(6)}$ type despite $S_{(6)}$ having the highest C:N ratio (Table 1).

In all cases, treatments with *long residues* resulted in higher final C:N ratio, C, and N content than those of the same type with *short residues* at the end of the growing season. Higher final C:N ratio in residues of the same type denotes lower decomposition rate, because C:N ratio is decreasing during decomposition after an initial short increase (Spain and Hodgen, 1994). Considering the dry mass loss of residues was not significantly affected by their size, this is clear evidence that *long residues* contributed to a higher accumulation of C and N on soil surface, in the form of C and N content retained in residues, at the end of the growing season than the *short residues.* This suggests possible positive effects on the mineralization of C and N, and possibly of other nutrients, for subsequent crops with continuous application of long versus short residue mulches (higher fertilization capacity).

Moreover, the final C:N ratio was highly affected by residue quality. Decomposition drive to lowering of the C:N ratios of the different residue types with a tendency to equalize (Akratos *et al.*, 2017). However, the lignin and other recalcitrant fractions of residues that were less affected during decomposition (Jensen, 2014) resulted in higher final C:N ratios of residues. Indeed, final C:N ratio was higher at $P_{(1)}$ and $S_{(6)}$ types and especially at $PL_{(1)}$ and $SL_{(6)}$ treatments (*long* residues of higher recalcitrance) than at $B_{(12)}$ and $H_{(17)}$ and especially at $BS_{(12)}$ and $HS_{(17)}$ (short residues of lower recalcitrance) (Table 1). Recalcitrance was distinguished by initial NDF content since there were no significant differences in initial ADL (lignin) content of the residues. Besides, Jensen et al. (2005) found that hemicellulose and cellulose were the factors that explained the greatest variability of C mineralization rather than lignin. Therefore, C and N mineralization of residues were affected mainly by residue size and initial NDF content rather than by initial C:N ratio and lignin content. It is possible that lower final N content in *short residues* was due to slightly increased N availability from more recalcitrant fractions, caused by chopping, and subsequent N uptake by plants or N losses by leaching. Chopped material is decomposed faster during the early days or weeks of decomposition because of larger exposure of the residue surface to direct contact with soil and soil microbes (Jensen, 2014). Therefore, the final residue quality was affected by both residue size and residue quality.

Moreover, the lack of correlation or the weak correlations between final C and N of treatments with *long residues* versus the strong relevant correlations in *short residues* may be an indication that different groups of microbes were responsible for the decomposition in each case. Assuming that the *short residues* are in better contact with the soil, the decomposition of *short residues* should be dominated by bacteria, and those of *long* residues by hyphae-producing fungi (Grosso et al., 2016; Abiven & Recous, 2007) . Bacteria preferentially decompose the readily available fractions leaving the more recalcitrant lignin fraction, while fungi decompose the more recalcitrant fractions (Strickland and Rousk, 2010). Thus, the correlation index (r) between C and N of final residues could possibly be used to estimate the degree and the microbial pathway of decomposition of mulch residues.

Dry mass loss was not significantly affected by residue size in the long term, which corresponds to previous findings (Ambus et al., 2001; Bremer et al., 1991). However, it seems to be related to both residue recalcitrance and C:N ratio or N content. This was confirmed by the simple linear regression analysis where the influences of NDF, N, and C:N ratio on residue mass loss were statistically significant, although each one of the three independent variables could explain only a small part of the variation of the dry mass loss (from 21.12 to 24.50%). Decomposition of lignin in residues typical for high recalcitrance and high N content is restricted because the relevant decomposer microbes need a readily available C source. Excessive N makes these microbes more vulnerable to competition with other microbes (Fog, 1988). Therefore, the higher the initial NDF content of residues the lower the residue dry mass loss (primarily), and the higher the initial N content in residues of higher recalcitrance the lower the final dry mass loss. Likewise, Xu et al. (2017), in a one-year experiment, noticed significant differences in biomass loss, higher in species with higher C:N ratio but low recalcitrance (polyphenol, lignin, and tannin) in residues cut to less than 2 cm and buried at 10 cm depth in sealed litter bags. The microorganisms adapted to the decomposition of recalcitrant substances are favoured by residues with a higher C:N ratio as they are able to outcompete other types of microbes (Bremer et al., 1991). Alternatively, higher diversity of residues may trigger higher diversity of decomposer microbes and consequently higher specificity in enzymatic activity during decomposition (Fontaine et al., 2003), driving to higher residue mass loss. Shu et al. (2021) also observed higher microbial activity in residue mulch of higher diversity (mixed than single plant species), but not higher microbial biomass or microbial community composition, and attributed this effect to the activation of dormant microbial populations by residue mixtures.

The fact that long residues resulted in higher final C and N content than short residues, while at the same time residue mass loss was not significantly affected by residue size, implies that long residues maintained higher fertilization capacity than short residues at the end of the growing season, in line with our hypothesis.

Dynamics of soil nutrients

At and after barley growing stage GS61 (Tottman *et al.*, 1986), the N uptake requirement of barley plants declines as plant growth has almost been completed and plants redistribute N to feed the developing grains (YARA, 2021). The decomposition of the residues initially affected the availability of NH_4^+ and soluble organic N (SON). NH_4^+ is typically not prone to leaching (Brady and Weil, 2002), but may be subjected to nitrification (Justes *et al.*, 2012) and the resulting NO_2^- and NO_3^- which may leach downwards – a process detected in our samples from the 50-55 cm depth on day 70. Subsequently, the nitrification activity was restricted considerably on day 137 probably due to cessation of irrigation to promote seed maturity (Jalota *et al.*, 2018). Both *short* and *long residues* led to significantly higher values of soil NH_4^+ on day 137 than on day 70 (Tables S8, S9, and Fig S4). It is possible, the observed lower values of soil NH_4^+ on day 70 to be attributed to plant uptake because maximum nutrient uptake is up to GS70 (end of flowering stage) (Malhi et al., 2007). Moreover, mean values of *short residues* were higher than those of *long residues* on day 70 and the opposite was true on day 137, although differences were not significant (Table S8). This shows that the rate of N mineralization was initially higher in *short residues*, but it was reversed in favour of long residues sometime up to day 137. This is supported by work from, Angers and Recous (1997) who observed initially higher decomposition rates at small particle size residues incorporated into soil, followed by higher rates at long size (up to 10 cm) one. In contrast, experiments with fine and ground (usually <1 cm) particle size residues mixed with soil showed effect of particle size on N dynamics only in early stage of decomposition (Bremer et al., 1991; Ambus and Jensen, 1997; Ambus et al., 2001).

Mean values of soil NO_3^- were not significantly affected by the treatments, although they were far lower than the initial measurements in bulk soil. In addition, no significant effect of residue diversity or functional traits on soil NH_4^+ content was observed on both day 70 and day 137. Contrary to our experiment, N mineralization was reported to be affected by residue chemical composition after 100 days of incubation, where high positive correlations had been found between N mineralization and N to lignin content ratio of residues (Zhonglu *et al.*, 2015). Any such effect in our experiment possibly either occurred at earlier stages of decomposition and differences were soon compensated or it was negligible. Moreover, Fox et al. (1990) concluded that when incorporating residues into soil, (lignin + polyphenol):N ratio could be used as a reliable predictor of N mineralization rate of residues, but they did not find significant correlation between N mineralization and residue N or lignin or polyphenol content alone.

Soil K was higher in all treatments with residues than in *Control*, in concordance with previous research (Singh and S. Khind, 1992). The return of plant residues to the soil is a considerable source of soil K replenishment. K is readily released as K^+ ions to soil solution during decomposition because, it remains in plants in ionic form in the cell solution and contributes, like Mg, to the production of extracellular enzymes (Xie et al., 2020). We hypothesised that the residue chemical composition (quality) as well as residue size would affect nutrient dynamics. Our data showed that K was the only nutrient that was

significantly affected by residue chemistry. Soil K was higher in residues with higher C:N ratio, and this positive correlation was statistically significant as it was confirmed by Spearman's correlation. Probably, the fact that K largely remains in the plant cells in ionic form makes it less prone to be bound in residue recalcitrant substances and therefore its release to soil solution during decomposition is dependent on initial residue C:N rather than NDF content. It is possible, residues of higher quality released higher amounts of K earlier due to higher initial rates of decomposition, while the opposite was true with lower quality residues (Campbell *et al.*, 1993).

Soil micronutrients Fe, Mn, Zn, and Cu had significantly higher mean values at 0-5 cm depth than at 20-25 cm. Normally their concentrations are higher at surface (Ap) soil horizons (Fageria et al., 2002). Soil Zn was the only nutrient that was significantly affected by residue size, in confirmation to our hypothesis, with higher values in *short residues*, but likewise K, only on day 137 at 0-5 cm depth. It is estimated that about 50% of soils cultivated with cereals worldwide suffer from low Zn content with negative impact in production and grain quality (Graham and Welch, 1996). Increased release of Zn at later stage of decomposition by *short residues* may be deemed as a precursor of an increased Zn release by *long residues* in a successive cash crop. Contrary, higher soil Zn content could be the result of phytosiderophore exudates by barley plants (graminaceous monocotyledonous species) in Fe or Zn deficient soil (Li *et al.*, 2014). In this case *long residues* provide an advantage by slow and stable release of micronutrients avoiding Zn deficiency.

Principal Component Analysis showed that *Control* was generally a negatively related treatment with all soil nutrients in comparison to the other treatments. This indicates the value of residue mulch in enrichment of soil nutrients even at 20-25 cm depth on both day 70 and day 137.

Impact on barley plants

Yield of barley plants was not affected by residue diversity or size. This is consistent with earlier studies, e. g. Reichert et al. (2015) found no differences in crop (cassava) yield between treatments with chopped mulch, although it concerned residues with very small particle sizes. On the contrary, Awopegba et al. (2017) noticed significant differences in crop (maize) yield between types of treatments with chopped material applied on soil with a traditional hoe. In our experiment, there were indications that *long residues* resulted in higher seed protein content than *short residues*. L*ong residues* have slower rate of decomposition and are thus able to provide more N at later stage of decomposition (GS37, Tottman *et al.*, 1986) than the *short residues*, which is crucial to increase protein content of grains (Boyle, 2017). Indeed, *long residues* resulted in significantly higher rates of barley stem elongation 42 days after plant emergence (at GS31 to GS39 – rapid stem elongation stage) in comparison to *short residues*. This is an indication of increased N supply by long residues at the stage of flag leaf emergence (GS37) which results in increased seed protein content, because N uptake by plants at GS37 is transferred to ear and seed development at GS59 to GS87 (Boyle, 2017). The

association of the main stem elongation rate on day 42 after plant emergence with the final seed protein content was confirmed by the linear regression analysis which showed a small but significant influence. In addition, this correlation was further confirmed by Spearman correlation which showed a significantly negative correlation between final seed protein content and length of ears. Therefore, it seems that *long residues* contributed to higher main stem elongation rate on day 42 after plant emergence resulting in shorter ear length and higher seed protein content opposite to the *short residues*. Contrary, mulch diversity was not found to impact protein content. The effect of residue size on grains' protein content should be further investigated as it is of great interest for both farmers and food processors.

In line with our hypothesis, it has already been shown that *long residues* had higher final C:N ratios, C, and N in comparison to *short residues*. In addition, it has been shown that there were no significant differences in residue dry mass loss or in crop yield between different residue size. Therefore, we reach the conclusion that *long residues* are characterized by an enhanced potential to provide nutrients to soil microbes and to the next crop at the end of the growing season than the *short residues*, without a yield penalty. At the same time, *long residues* provide better coverage of soil surface with organic material. Considering results were derived from a one growing season incubation experiment, it is highly possible the iteration of the practice of using *long residues* as mulch in successive crops could result in continuous enrichment of soil with nutrients, increase of soil organic matter, better physical conditions on soil surface, improvement of soil microbial community, and higher cost effectiveness in comparison to *short residues*. Further long-term research is needed to confirm this hypothesis.

The AMF root colonization was not significantly different between treatments. However, mean values were increased with increasing number of residue plant species. Regression analysis, considering the species richness (17, 12, 6, 1 and 0) of the different residue types and *Control*, showed a statistically significant influence of species richness in AMF root colonization, although the predictor could explain only a small variation of the response. Nevertheless, the fact that an increase in plant residue species richness on the soil surface is responsible for even a small increase in AMF root colonization of the crop plants is very important in terms of agricultural economic performance. This was consistent with previous finding by Burrows and Pfleger (2002) who observed increasing AMF sporulation with increasing number of species of cover crop plants, which was attributed to increased number of AMF species triggered by the increased cover crop plant diversity. In our experiment the potential increase in AMF colonization in barley plant roots was triggered plainly by the deposition of cover crops cultivated elsewhere. This was an indication that residue mulch diversity may influence AMF root colonization and possibly the soil microbial community in general, which is in accordance with other researchers' observations (Bainard et al., 2011; Fontaine et al., 2003; Wu et al., 1993). Nevertheless, it seems that the influence of residue diversity on soil microbial activity is greater at the early stage of decomposition due to functional complementarity in microbial communities which reduces

competition in comparison to residues of lower diversity (Shu et al., 2022), but further investigation is needed.

Conclusions

Dry mass loss of plant residue mulch was significantly affected by residue chemical composition. It was higher in residues of lower NDF and with lower N content. Moreover, the final quality of residues was highly affected by residue size, resulting in higher fertilization capacity of *long size* residues than of *short size* one. Soil K and Zn content were found to be significantly affected by residue quality and size, respectively, at later decomposition stages. Treatments of higher initial C:N ratio provided higher amounts of soil K, while *short residues* provided more Zn. Crop yield was not affected by residue quality or size. *Long residues* supported significantly higher rates of barley stem elongation than *short residues* at the stage of rapid stem elongation where N availability is determined for a high seed protein content. There were indications that mulches with *long residues* increased seed protein content, which is a key result important for both farmers and grain processors. The Arbuscular Mycorrhizal Fungi root colonization was higher but not significantly different in treatments with higher plant species richness, which indicates a possible effect of diverse mulch in soil microbial community. In summary, *long residue* mulches composed of diverse mixtures of plant species have enhanced residual fertilization capacity at the end of the growing season than short residues with no deleterious effect on crop yield. Further long-term research is needed to investigate the effect of continuous application of plant residue mulches on the enrichment of soil nutrient content, increase of soil organic matter, and improvement of soil microbial diversity.

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Fig. S1 a) the back side of the rhizotrons settled at the outdoor, b) the front side covered with aluminum foil, c) a view from above with the growing plants, d) rhizotron with the glass removed to allow sampling.

Table S1 Diverse forage mixture species selection list (P: Perennial Ryegrass; S: Smart Grass; B: Biomix; H: Herbal)

Species	**Latin**	**P**	**S**	**B**	**H**
Perennial Ryegrass	*Lolium perenne* L.	✓	✓	✓	✓
Timothy	*Phleum pratense* L.		✓	✓	✓
Cocksfoot	*Dactylis glomerata* L.			✓	✓
Festulolium	-			✓	✓
Tall Fescue	*Festuca arundinacea* Schreb.				✓
Meadow Fescue	*Festuca pratensis* Huds.			✓	✓
Red Clover	*Trifolium pratense* L.		✓	✓	✓
White Clover	*Trifolium repens* L.		✓	✓	✓
Alsike Clover	*Trifolium hybridum* L.			✓	✓
Sweet Clover	*Melilotus* spp.				✓
Black Medick	*Medicago lupulina* L.			✓	
Lucerne	*Medicago sativa* L.			✓	
Sainfoin	*Onobrychis spp.*				✓
Birdsfoot Trefoil	*Lotus corniculatus* L.				✓
Plantain	*Plantago lanceolata* L.		✓	✓	✓
Chicory	*Cichorium intybus* L.		✓	✓	✓
Yarrow	*Achillea millefolium* L.				✓
Burnet	*Sanguisorba minor* Scop.				✓
Sheep's Parsley	*Petroselenium crispum* Mill.				✓

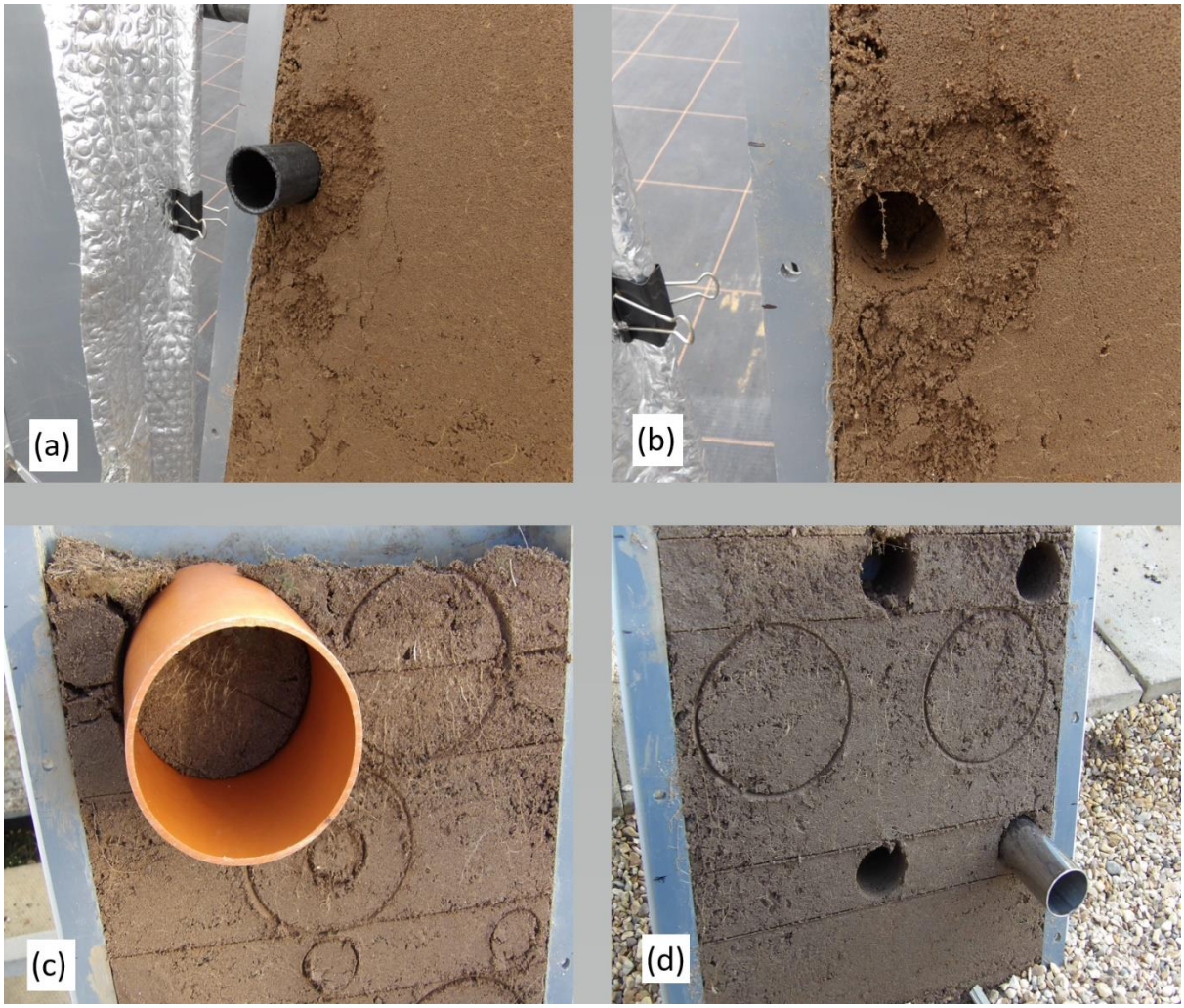

Fig. S2 a) and b) 1st sampling period (from the left side of the rhizotrons), c) and d) 2nd sampling period.

Table S2 Tukey's post-hoc test for significant differences (p-value < 0.05) of initial C:N ratio, % C, and % N content of plant residues between different types. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species)

C:N ratio			**% C**			**% N**		
Difference of Levels	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
P - B	-5.05	<0.001	*P - B*	3.85	0.003	*P - B*	5.38	<0.001
P - H	-3.14	0.020	*P - H*	4.06	0.002	*P - H*	3.90	0.003
S - H	4.40	0.001				*S - H*	-3.50	0.008
S - P	7.54	<0.001				*S - P*	-7.39	<0.001

Table S3 Initial C:N ratio, % C, and % N content for the different types of residues (N = 8). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species)

Variables	**C:N ratio**		**% C**		**% N**	
Types	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	30.420	3.640	41.229	0.256	1.373	0.178
H	28.069	1.801	41.207	0.195	1.473	0.091
P	24.199	2.268	41.644	0.160	1.734	0.170
S	33.502	1.650	41.400	0.237	1.238	0.055

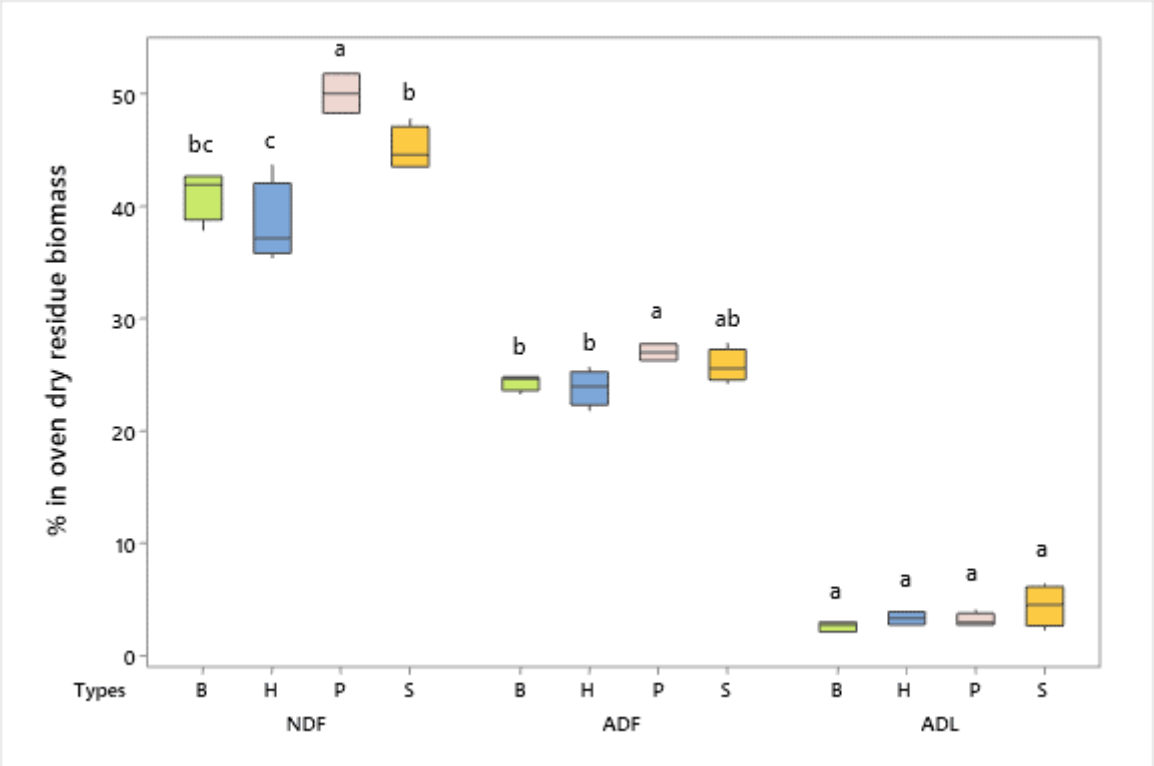

Fig. S3 Box and whiskers plots of plant residue initial % NDF, % ADF, and % ADL for the different residue types. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin). Types that do not share a common letter are significantly different ($p < 0.05$)

Table S4 Initial NDF, ADF, and ADL content (%) of the different residue types (N = 4). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin)

Variable	**%NDF**		**%ADF**		**%ADL**	
Type	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	41.160	2.230	24.359	0.708	2.616	0.451
H	38.360	3.640	23.857	1.575	3.333	0.607
P	50.053	1.893	27.027	0.764	3.161	0.582
S	45.084	1.932	25.798	1.472	4.441	1.821

Table S5 Tukey's post-hoc test for significant differences (p-value < 0.05) of initial % NDF, ADF, hemicellulose, and cellulose content of residues between the different types of residues. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin)

% NDF				**% ADF**			
Difference of Levels	**Difference of Means**	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**Difference of Means**	**T-Value**	**Adjusted P-Value**
P - B	8.90	4.98	0.002	*P - B*	2.66	3.15	0.036
P - H	11.69	6.55	<0.001	*P - H*	3.17	3.75	0.013
S - H	6.72	3.76	0.012				
S - P	-4.97	-2.78	0.069				
% Hemicellulose				**% Cellulose**			
Difference of Levels	**Difference of Means**	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**Difference of Means**	**T-Value**	**Adjusted P-Value**
P - B	6.77	5.98	<0.001	*P - H*	3.34	2.57	0.099
S - B	4.31	3.81	0.012				
P - H	8.35	7.37	<0.001				
S - H	5.89	5.20	0.001				

Table S6 Tukey's post-hoc test for significant differences (p-value < 0.05) of final C:N ratio, % C, and % N content of residues between treatments (*HL, HS, BL, BS, SL, SS, PL, PS*). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (short of 1.5 cm *(S),* and long of 30cm *(L)*)

C:N ratio			% C			% N		
Difference of Levels	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
PL - BL	3.01	0.093	HL - BS	3.61	0.026	*BS - BL*	-3.24	0.058
PL - BS	4.22	0.006	PL - BS	4.15	0.007	*HS - BL*	-3.56	0.029
PS - BS	3.11	0.076	SL - BS	4.04	0.010	*PS - BL*	-3.69	0.021
SL - BS	3.26	0.055	HS - HL	-3.60	0.027	*HL - BS*	3.27	0.055
PL - HS	3.78	0.017	PL - HS	4.14	0.008	*SL - BS*	3.00	0.096
			SL - HS	4.03	0.010	*HS - HL*	-3.59	0.027
			PS - PL	-3.10	0.078	*PS - HL*	-3.73	0.020
			SL - PS	2.99	0.097	*SL - HS*	3.32	0.049
						SL - PS	3.46	0.036

Table S7 Final C:N ratio, % C, and % N of the different treatments (*HL, HS, BL, BS, SL, SS, PL, PS*) (N = 4). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (short of 1.5 cm *(S),* and long of 30cm *(L)*)

Variables	C:N ratio			% C		% N	
Treatments	**Mean**	**StDev**	**Initial – final C:N**	**Mean**	**StDev**	**Mean**	**StDev**
BL	14,690	2,320	15.7	27.90	4.83	1.897	0.103
BS	13,079	0,651	17.3	17.02	3.65	1.303	0.279
HL	16,180	2,250	11.9	30.73	4.83	1.903	0.216
HS	13,666	0,281	14.4	17.07	5.16	1.244	0.356
PL	18,700	2,960	5.5	32.79	4.30	1.762	0.121
PS	17,221	1,824	7.0	21.01	4.28	1.218	0.193
SL	17,422	1,652	16.1	32.37	4.95	1.853	0.173
SS	16,150	1,607	17.3	22.65	9.10	1.385	0.442

Table S8 Significant differences in soil available NH_4^+ content (mg/kg of oven dry soil) in samples taken on day 70 and on day 137 after mulch application, from 5-10 cm and from 50-55 cm depths, and two plant residue fibre sizes (short of 1.5 cm *(S),* and long of 30cm *(L)*) (N = 64)

Factors	Factor levels	Mean	StDev	F-value	P-value	Sign. diff.
Time	day70	1.302	0.356	46.65	<0.001	a***
	day137	1.678	0.379			b***
Depth	10	1.689	0.280	52.13	<0.001	a***
	55	1.292	0.428			b***
Time*Size	day70 *L*	1.248	0.321	3.00	0.087	a
	day70 *S*	1.357	0.385			a
	day137 *S*	1.638	0.345			b
	day137 *L*	1.719	0.412			b

*Combinations not shown in the table are not significant with all the others. Mean values that do not share a common letter are significantly different between them. Means with *** are very highly significant different, with ** are highly significant different, with * are significant different, with no * are nearly significant different*

Table S9 Tukey's post-hoc test for significant differences (p-value < 0.05) of soil NH_4^+ content for Size*Time interaction for *long* (*L*) and *short residues* (*S*), for day 70 and for day 137 after mulch application, from both 5-10 and 50-55 cm depths

Difference of Size*Time Levels	T-Value	Adjusted P-Value	Difference of Size*Time Levels	T-Value	Adjusted P-Value
(*L* day137) - (*L* day70)	6.05	<0.001	(*S* day70) - (*L* day137)	-4.65	<0.001
(*S* day137) - (L day70)	5.01	<0.001	(*S* day137) - (*S* day70)	3.61	0.003

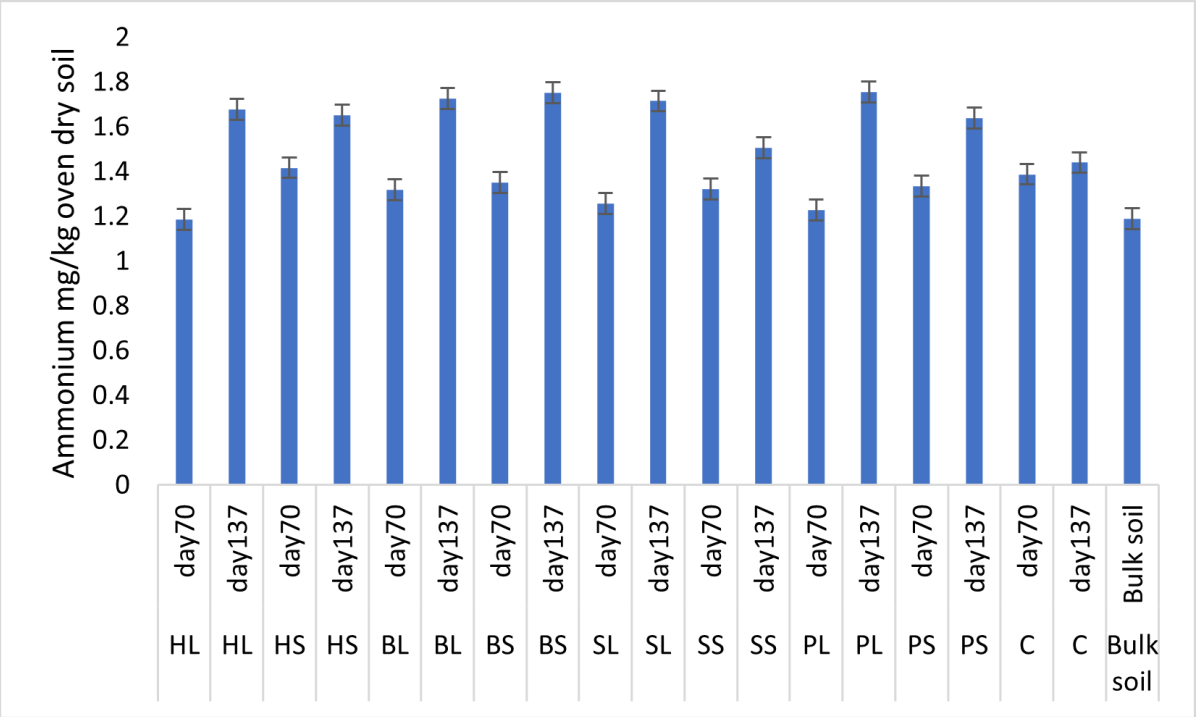

Fig. S4 Soil NH_4^+ content (mg/kg of oven dry soil) from the different treatments (*HL, HS, BL, BS, SL, SS, PL, PS, and Control*) at both 5-10 and 50-55 cm depths, in September (day 70) and in November (day 137 after mulch application). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (short of 1.5 cm *(S),* and long of 30cm *(L)*). Control treatment (*C*) was with no residues. Means and bars of one standard error from the mean are depicted. The bulk soil value is also quoted for comparisons

Table S10 Soil available macronutrient (P, K, and Mg) contents (mg/kg of oven dry soil) in samples taken on day 70 and on day 137 after mulch application, from 0-5 cm and from 20-25 cm depths

Element	Factors	Factor levels	N	Mean	±SD	F-value	P-value	Sign. diff.
P	Time	day70	64	387.415	26.612	3800.71	<0.001	a***
		day137	64	210.224	162.204			b***
	Depth	25	64	388.441	26.590	3889.23	<0.001	a***
		5	64	209.198	161.117			b***
K (fi)	Time	day70	64	103.163	37.343	28.40	<0.001	a***
		day137	64	79.907	21.985			b***
	Depth	5	64	97.985	42.640	8.74	0.004	a**
		25	64	85.084	15.821			b**
Mg (tr)	Time	day70	64	66.096	14.648	48.11	<0.001	a***
		day137	64	58.259	11.703			b***
	Depth	25	64	72.640	10.606	372.26	<0.001	a***
		5	64	51.715	6.902			b***

*Combinations not shown in the table are not significant with all the others. Mean values that do not share a common letter are significantly different between them. Means with *** are very highly significantly different, with ** are highly significantly different, with * are significantly different, with no * are nearly significantly different. Where tr = results obtained after transformation of data to log_{10} or to optimal or rounded λ, fi = results obtained without transformation of data and further investigation is needed because normality and/or equality of variances of data were not satisfied even after transformation*

Table S11 Significant differences in soil available micronutrient (Fe, Mn, Zn, and Cu) contents (mg/kg of oven dry soil) in samples taken on day 70 and on day 137 after mulch application, from 0-5 cm and from 20-25 cm depths (N = 64)

Element	Factors	Factor levels	Mean	±SD	F-value	P-value	Sign. diff.
Fe	Depth	5	306.880	16.924	589.62	<0.001	a***
		25	246.376	10.859			b***
Mn	Depth	5	52.568	2.838	107.49	<0.001	a***
		25	46.414	3.528			b***
Zn (tr)	Time	day70	26.301	15.841	46.91	<0.001	a***
		day137	20.167	13.065			b***
	Depth	5	26.807	14.200	66.21	<0.001	a***
		25	19.661	14.590			b***
Cu (fi)	Time	day137	5.199	2.363	23.94	<0.001	a***
		day70	4.322	1.320			b***
	Depth	5	6.280	1.723	287.38	<0.001	a***
		25	3.240	0.244			b***

*Combinations not shown in the table are not significant with all the others. Mean values that do not share a common letter are significantly different between them. Means with *** are very highly significantly different, with ** are highly significantly different, with * are significantly different, with no * are nearly significantly different. Where tr = results obtained after transformation of data to log_{10} or to optimal or rounded λ, fi = results obtained without transformation of data and further investigation is needed because normality and/or equality of variances of data were not satisfied even after transformation*

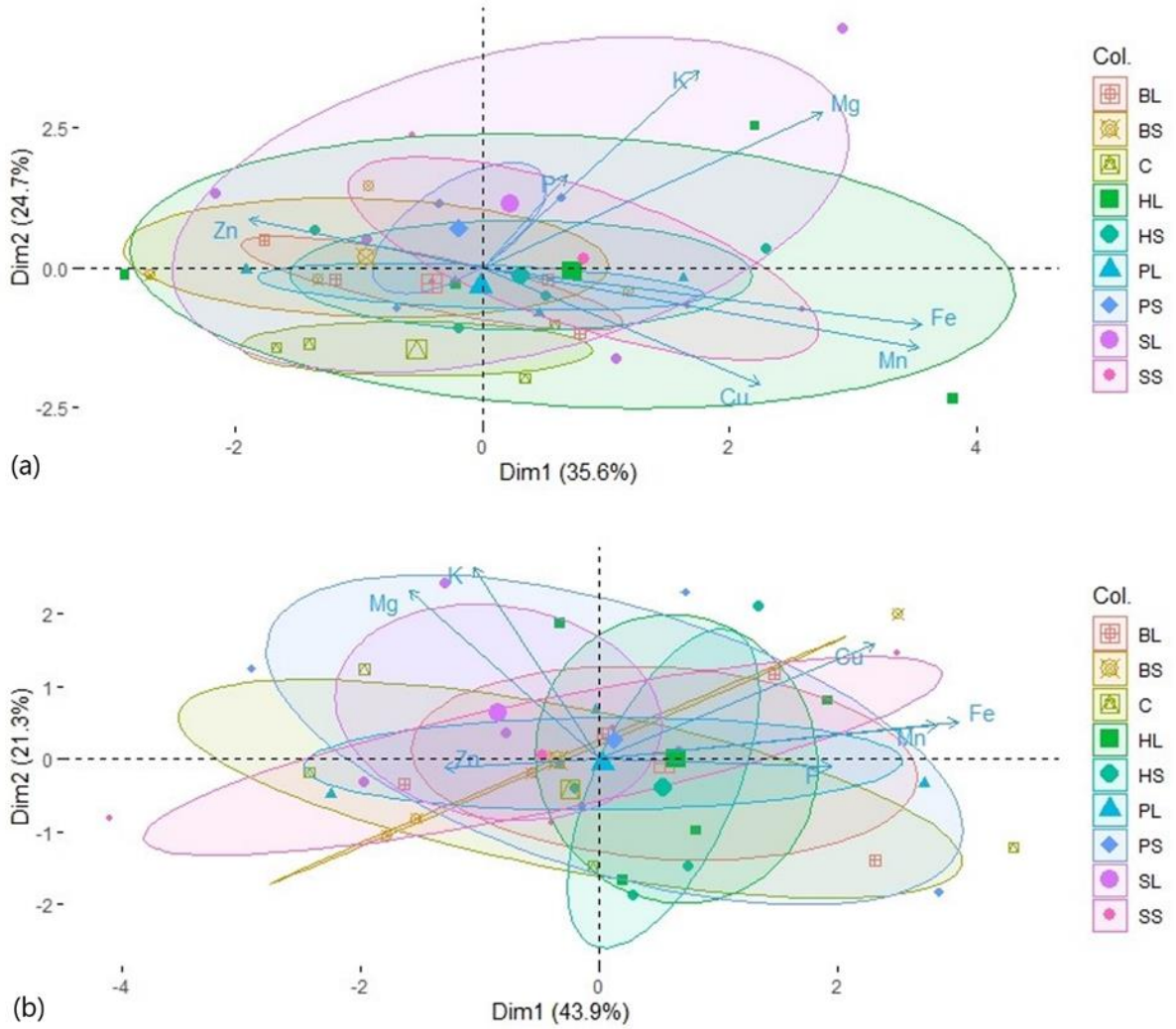

Fig. S5 PCA ordination of nutrients P, K, Mg, Fe, Mn, Zn, and Cu as affected by the treatments *HL, HS, BL, BS, SL, SS, PL, PS,* and *Control (C)* on day 70 after mulch application (a) at 0-5 cm, and (b) at 20-25 cm depth. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (*short* of 1.5 cm *(S),* and *long* of 30cm *(L)*). Control treatment (*C*) was with no residues. Large points depict mean values, while ellipses depict confidence intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1 normalized score, and left axis represents Dim2 normalized score

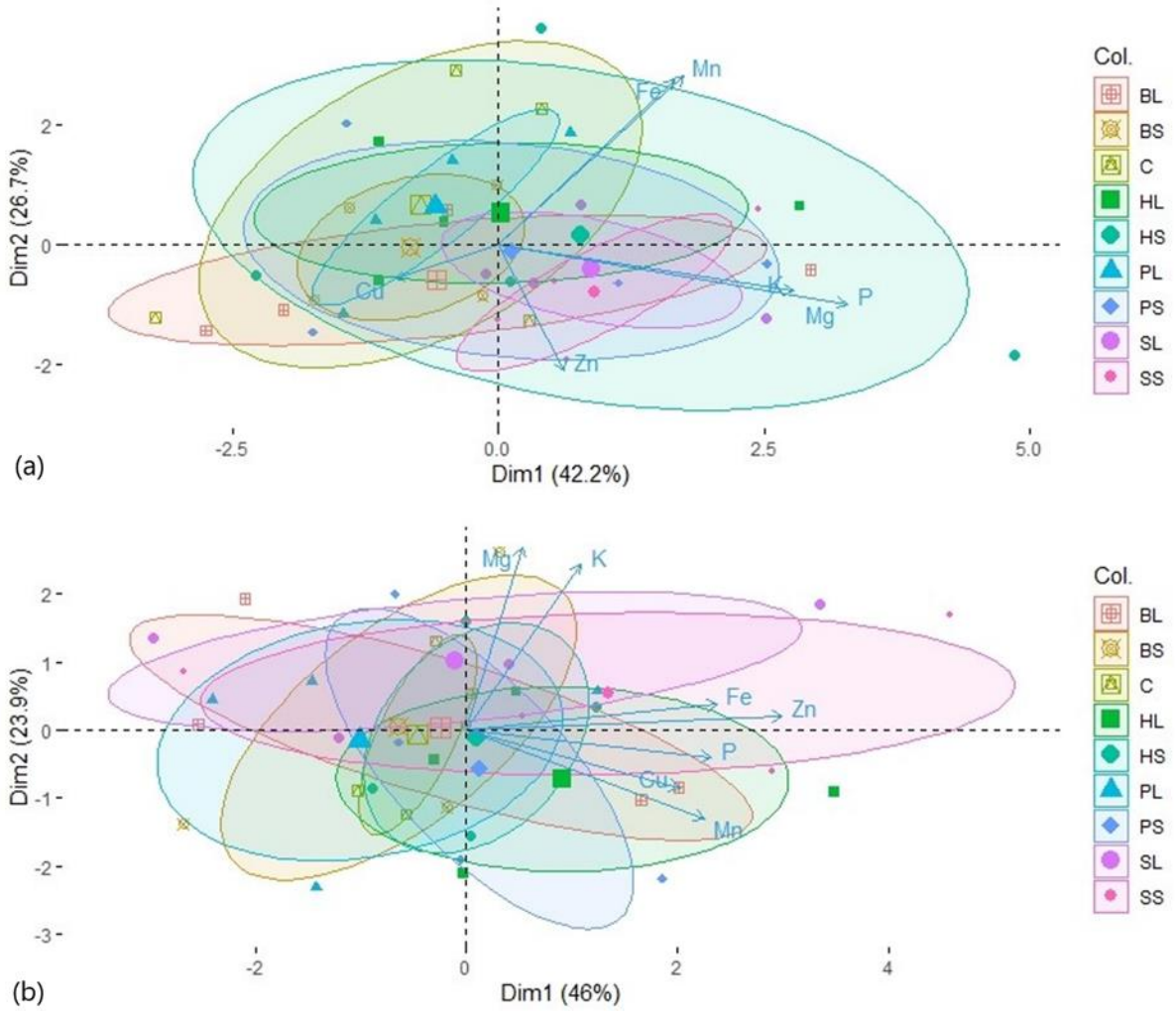

Fig. S6 PCA ordination of nutrients P, K, Mg, Fe, Mn, Zn, and Cu as variables for the treatments *HL, HS, BL, BS, SL, SS, PL, PS,* and *Control* (*C*) concerning measurements (a) at 0-5 cm, and (b) at 20-25 cm depth, on day 137 after mulch application. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (*short* of 1.5 cm *(S),* and *long* of 30cm *(L)*). Control treatment (*C*) was with no residues. Large points depict mean values, while ellipses depict confidence intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1 normalized score, and left axis represents Dim2 normalized score

Table S12 Barley seed protein content of the different treatments (*BL, BS, C, HL, HS, PL, PS, SL, and SS*) (N = 4). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Residue treatments including four plant diversity mixtures (types) and two plant residue fibre sizes (short of 1.5 cm *(S),* and long of 30cm *(L)*). Control treatment (*C*) was with no residues (N = 4)

Treatment	Mean	StDev	Treatment	Mean	StDev
BL	7.179	0.264	PL	6.930	0.234

BS	6.908	0.212	PS	7.087	0.691
C	6.995	0.545	SL	7.314	0.061
HL	7.877	1.271	SS	7.193	0.155
HS	7.126	0.480			

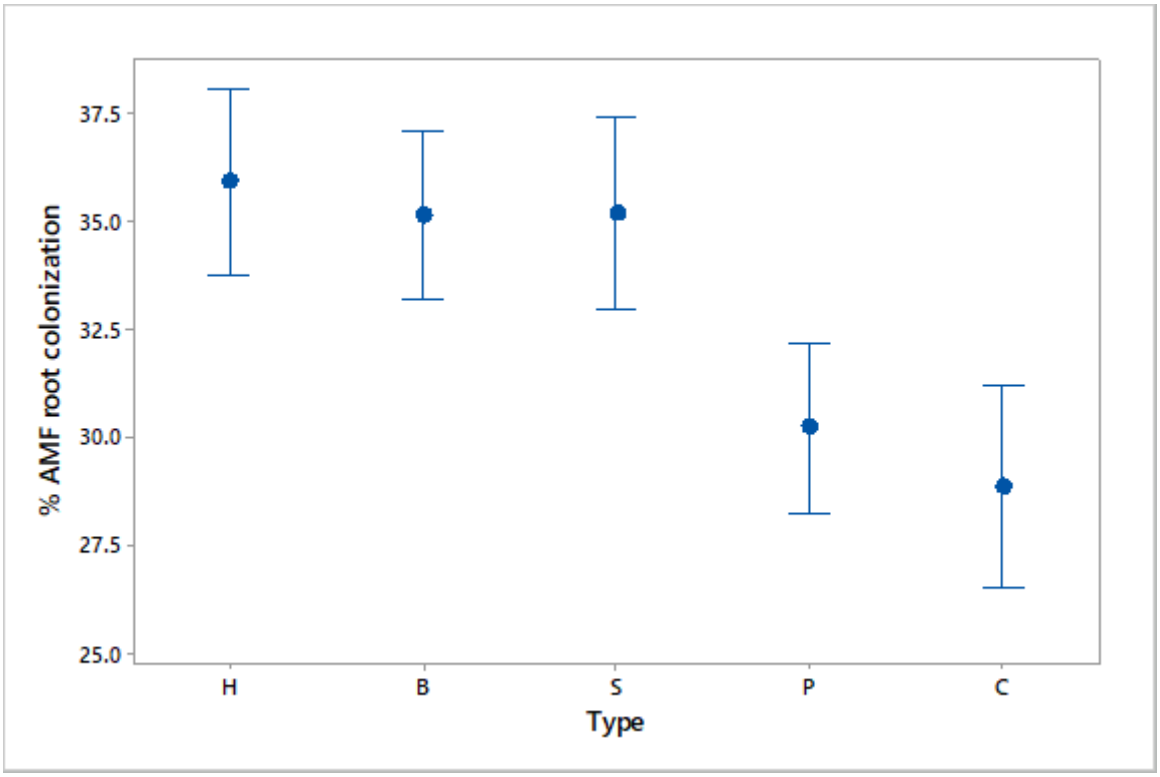

Fig. S7 Interval plot of AMF root colonization (%) in all residue types and *Control* 137 days after mulch application (N = 8). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), and Herbal ***(H)*** (17 species). Means and bars of one standard error from the mean are depicted

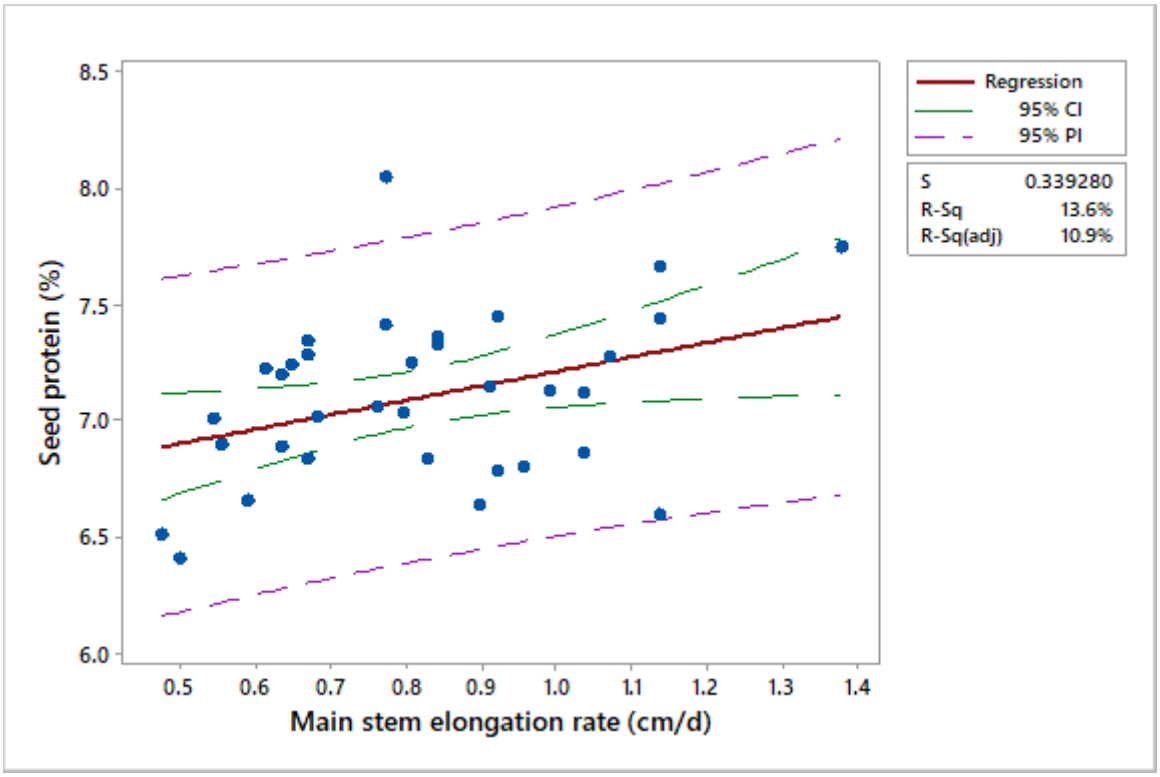

Fig. S8 Fitted line plot of linear regression analysis of seed protein content (%) at harvest time with main stem elongation rate (MSER) (cm d^{-1}) of barley plants on day 42 after plant emergence (red line). Confidence interval is enclosed by green dashed lines, and prediction interval by purple dashed lines. Confidence level = 95.0. Regression equation: Seed protein (%) = 6.589 + 0.6228 MSER

Chapter 3

Paper 2

Long stemmed diverse plant-based mulches are promising fertilizers influencing soil microbial communities at later stages of cultivation period.

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Authors' contributions: Conceptualization, D.G.; Experimental design, D.G., and M.L.; Experimental work, D.G.; Laboratory analyses, D.G.; Data analysis, D.G.; Data curation, D.G., M.L., and M.T.; Supervision, M.L., and M.T.; Writing – original draft, D.G.; Writing – review and editing, D.G., M.L., and M.T.

Key words: Plant-based mulch, long residues, residue mixtures, residue quality, soil nutrients, soil microbes, yield, later stages of decomposition.

Abstract

The benefits of plant-based mulch are not restricted to improvement of soil physical properties, but it also seems to be valuable as a fertilizer, and in maintenance of vigorous soil microbial communities throughout the cultivation period. However, most research in incubation experiments is focused on short-size residues and little attention has been given on whole plants. and their extended influence on soil fertility.

We investigated the effect of long-residue mulch quality on soil fertility, soil microbial biomass, crop yield, and seed quality. Samples were taken 69 and 195 days after mulch application. Treatments included residue mixtures of 17, 12, 6, and 1 plant species, wood chips, and a control without residues, applied in rhizotrons planted with barley.

High-quality residues resulted in significantly higher residue mass loss than residues of lower quality. On day 69 soil microbial biomass was higher in high-quality residues, opposite of day 195. Fungal: bacterial ratio was higher on day 195 than on day 69 in all treatments. High-quality residues demonstrated higher total soil N than low-quality residues, on both days. Soil K was increased significantly in treatments with residues than in *Control*. Barley seed quality was significantly affected by residue quality, but not yield.

Generally, long-residue mulch quality can affect soil nutrient content and crop quality. However, its influence on soil microbial biomass at later stages of the cultivation period, and on crop quality is slow and not always detectable. Further research is necessary with continuous application of diverse long-residue mulch to investigate its behaviour on chemical and biological soil properties.

Introduction

The effect of plant-based mulch on soil physical properties are well recognized (Prats *et al.*, 2017; Donjadee and Tingsanchali, 2016; Jordán *et al.,* 2010; Blanco-Canqui et al., 2011; Mulumba and Lal, 2008), but that on soil chemical and biological properties have not been thoroughly studied.

Most of published research on soil chemical properties is focused on mulch contribution to C, N, and P mineralization, and in less extent on other nutrients. Significant differences in C mineralization have been found between different amounts of mulch application in various environmental conditions (Dietrich et al., 2017). Mulch of sugarcane residues increased soil organic carbon by 16% at 0-10 cm depth in a 6 year period (Razafimbelo et al., 2006). Residues of high quality (low C:N ratio) resulted in lower N immobilization (Abiven and Recous, 2007), and higher dry mass loss (Donjadee & Tingsanchali, 2016). Straw mulch (high C:N ratio) resulted in higher N uptake by crop seedlings, but demonstrated lower soil N content after seedling stage, probably due to enhanced NH_3 volatilization, in comparison to bare soil (Su *et al.*, 2014). However, N availability can be limited soon after mulch application in soil poor in nitrate content (Siczek & Lipiec, 2011), especially for low quality residues. Soil inorganic P is rapidly subject to fixation by soil particles or is immobilized by soil microbes, therefore, its mineralization in organic form by plant residues is a significant source for plant uptake (Lynch & Brown, 2001). In an 11-year experiment, mulching, in comparison to bare soil or removal of plant residues after cutting, increased soil P and K content, although not significantly. The number of applications was also important. Higher concentrations of nutrients were noticed from 1 to 3 applications in ascending order (Pavlu, Gaisler, Hejcman, & Pavlu, 2016). Organic matter added in soil from plant residues can release soluble forms of Fe and Mn by affecting oxidation-reduction processes, and functioning as a chelating agent (Singh and Khind, 1992). Chen (1983) concluded that green manure had small effect on Zn and Cu soil content, but it can sufficiently increase soil Zn content and crop yield in a Zn-deficient soil. Although the effect of mulching on soil nutrients is not readily obvious, it is believed it could be a sustainable way to increase soil fertility, provided it is available in sufficient amounts (Awopegba et al., 2017), especially in organic farming where artificial fertilizers are prohibited, or in cases where they are not available.

Differences have been reported in crop yield and quality under mulch application. Straw mulch combined with micronutrients increased production economics of broccoli (Prasad, 2018). Yield and nutritional value of maize seeds were increased under sufficient amount of mulch application (Awopegba et al., 2017). Su et al., (2014) attributed an increase in oilseed rape yield under straw mulch

in maintenance of higher soil water content and lower soil temperature oscillation. In another research, straw mulch increased nodulation and nitrogenase activity by reducing soil compaction, and increased soybean seed protein content but had no effect on yield (Siczek & Lipiec, 2011).

Mulch has been found to contribute to enrichment of soil microbial communities. Soil microbial biomass, expressed in CO_2 evolution, was influenced by differences between treatments in chemical composition (Bremer et al., 1991). Soil fungal: bacterial ratio was higher under litter with higher C:N ratio or when soil solution pH was low irrespective of litter chemistry (Grosso et al., 2016), but bacteria were found to dominate soil microbial community in a long term continuous residue input experiment (Frasier et al., 2016). However, Wang et al. (2015) did not observe any significant difference in fungal: bacteria ratio after residue addition. In the same experiment, although soil microbial populations of all microbial taxonomic groups were higher under high quality residues, the differences were negligible at the end of the incubation.

Residues of mixtures of plant species gain raising attention by researchers as they are considered to provide more ecological services in comparison to single plant species. Besides, plants growing in a mixture is the most common situation in natural ecosystems. Mixtures can influence decomposition process positively (synergistic effect), negatively (negative effect), or equally than expected (additive effect) (Redin et al., 2014). The most commonly studied are bispecific mixtures of legumes and non-legume species. Legumes have been used due to their N fixation capacity and their ability to provide readily available N for plant uptake (Tribouillois, Cruz, Cohan, & Justes, 2015). Combined non-legumes usually consist of species capable to capture excessive N in soil solution preventing losses by leaching (White et al., 2017). Phosphorus or other nutrient mobilizing species can be used as cover crops to facilitate crop plants to acquire nutrients, both by root exudates (Li *et al.,* 2014) or through decomposition process. Equally important is the effect of mixtures on soil microbial community. Microbial populations are characterized by enzymatic specificity on decomposition process (Fontaine et al., 2003), therefore a mixture of plant species substrate is supposed to trigger an increase in microbial diversity.

Most of the research on plant residues concern coarse or fine residue particle size (< 2.5 cm) of a single plant species, incorporated into soil (Nguyen & Marschner, 2017). Residues initially decomposed faster, but no significant differences were observed after a period of time. In addition, residues applied on the soil surface had lower gross N immobilization than when incorporated into soil (Abiven & Recous, 2007). The process of N mineralization is slowing down by increasing the residue particle size (Singh and Khind, 1992). In particular, residues of longer particle size over about 30cm, or of whole plants cut once near the soil surface, applied as mulch decompose slower, and may retain considerable capacity as fertilizers for the next crop. Furthermore, the ongoing decomposition maintains the soil microbial activity until next crop is planted. Long size residues are more economic because they do not

require supplemental energy to be broken to smaller particles. At the same time they provide physical protection on soil for longer period due to smaller degradation.

In agricultural practice, mainly in organic farming or in agroforestry, farmers may use shredded tree branches and twigs as mulch. These residues are characterized by quite high C:N ratios (>40), and of high recalcitrance, resulting in initial N immobilization (Brust, 2019). They favour fungal growth instead of bacteria (Barreiro et al., 2016), but their effect on chemical and biological properties at later stages of decomposition have not been thoroughly investigated. Future research on the effect of long-residue mulch on chemical and biological soil properties, throughout its decomposition period, is necessary.

We studied the effect of long size plant-based residue mulch on soil chemical and biological properties at later stages of decomposition as well as on crop yield and quality. Treatments included mixtures of plant species of a single plant species or wood chips (shredded tree branches and twigs with their leaves), and a control treatment without mulch.

We hypothesized that (i) residues of higher quality (lower C:N ratio, higher N content, and lower recalcitrance) are initially decomposed faster than those of lower quality, but the rate of decomposition is reversed at later stages of decomposition, resulting in significant differences in soil microbial biomass, in nutrient dynamics, and in crop quality, but not in crop yield (because controversial effects on crop yield were reported in previous research), (ii) the remaining residues at the end of the growing season still maintain a fertilizer capacity which is higher in residues of lower quality.

Methodology

Experimental design and materials

The experiment was conducted at the Crop and Environment Laboratory of the University of Reading in UK, from June 2019 to December 2019. The experimental unit was rhizotron constructed from 0.5 cm PVC sheeting and filled with modified soil media to grow annual cereal plants (Fig. S1, and S2). One side of each rhizotron was consisted of removable clear acrylic to allow observation of root growth and periodical soil sampling. Apart from when under observation, this side was covered with thermawrap silver foil to avoid light penetration and to minimize temperature fluctuation. Each rhizotron had 1 m height to allow root growth at depth, 30 cm length, and 5 cm width. A layer of gravel c.1.5 cm deep was placed at the bottom to allow drainage, and the rest was filled up to 10 cm below the surface with commercially supplied (Castleton Suppliers) well homogenized loamy sand topsoil (bulk soil) sieved to 8 mm. Initially, soil solution pH was 7.3 ± 0,032 SD, and soil texture was 86.1% sand, 5.7% clay, and 8.2% silt. We utilized 30 rhizotrons for 5 treatments and a control, each in 5 replicates. The rhizotrons were kept at 70^{o} angle for the duration of the experiment, with the

transparent side facing downwards to impel the plant roots to be developed largely on it to facilitate their visual observation. Initially they were placed at the outdoor and from October in a greenhouse.

Spring barley plants (*Hordeum vulgare L., var. Westminster*) were sown in germination trays filled with the same experimental soil. Selected plants with height between 15 cm and 20 cm were randomly transplanted into rhizotrons on 11th July 2019, 17 days after plant emergence, one plant in each rhizotron in the middle (15 cm from each side). An automated irrigation system with two drippers per rhizotron was installed to maintain 55-60% Water Holding Capacity (WHC). Growth rate of the barley plant was recorded every 1 to 2 weeks and the plants were finally harvested 182 days after plant emergence.

Five biomass residue treatments were established as mulch on the soil surface in the rhizotrons on 11th June 2019 (30 days before barley transplanted into rhizotrons). A *Control* treatment *(C)* without residues was also included. The biomass residue treatments were comprising five plant diversity mixtures (Types). The residue Types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass™ ***(S)*** (6 species), Biomix™ ***(B)*** (12 species), Herbal™ ***(H)*** (17 species), and Wood chips (***W***) (Table S1). Therefore, the 6 treatments were: ***H, B, S, P, W, and C.*** These residue types were suitable to address our hypothesis because they included residue mixtures with different characteristics. Residue characteristics include both residue diversity and functional traits. Residue diversity may concern either the species composition or the species richness which is the number of species participating in a residue mixture. The functional traits of residues may concern either the chemical composition, which determines the residue quality, or the morphological features of residues (Santonja *et al.*, 2018; Hattenschwiler et al., 2011). Wood chips were consisted of shredded tree residues of branches and twigs with their leaves, collected from the Harris Garden from the University of Reading campus on 9th June 2019 and stored at 4^{o}C. Fresh plant residues of all other types were collected from field plots, planted with the above forage mixtures, on 4th June 2019 and stored at 4^{o}C until application. The labels $P_{(1)}$, $S_{(6)}$, $B_{(12)}$, and $H_{(17)}$ have been adopted where it was considered necessary to indicate the number of species participating in the different residue types collected from field plots. All residues were cut to about 30 cm long except wood chips which were already shredded up to about 10 cm and were placed into rhizotrons as fresh mulch in quantities corresponding to 23 g of dry mass. Not any kind of fertilization, artificial or organic, other than the mulch was applied during experiment.

Sampling protocol

Soil samples were taken from rhizotrons in two timepoints. The first timepoint was 69 days after mulch application when the emergence of inflorescence was completed for most of the plants and the flowering was beginning, growing stage (GS) 59-60 (Tottman et al., 1986). Three soil samples were collected this timepoint from the left side of each rhizotron facing the glass from the depths of 0-5, 20-25 and 50-55 cm (Fig. 1).

The second sampling timepoint was 195 days after mulch application. Soil samples were taken from the depths of 0-5, and 50-55 cm from the right side of the rhizotrons (Fig. 1). All soil samples were air-dried and sieved to <2 mm. Soil samples from 0-5 cm depth in both timepoints were separated into two parts. Samples from one part were sieved to <2 mm soon after their extraction, freeze-dried and stored to -20°C for PLFA analysis. The plant residues were collected and dried at 80°C for 48 hours to constant weight to estimate residue dry mass.

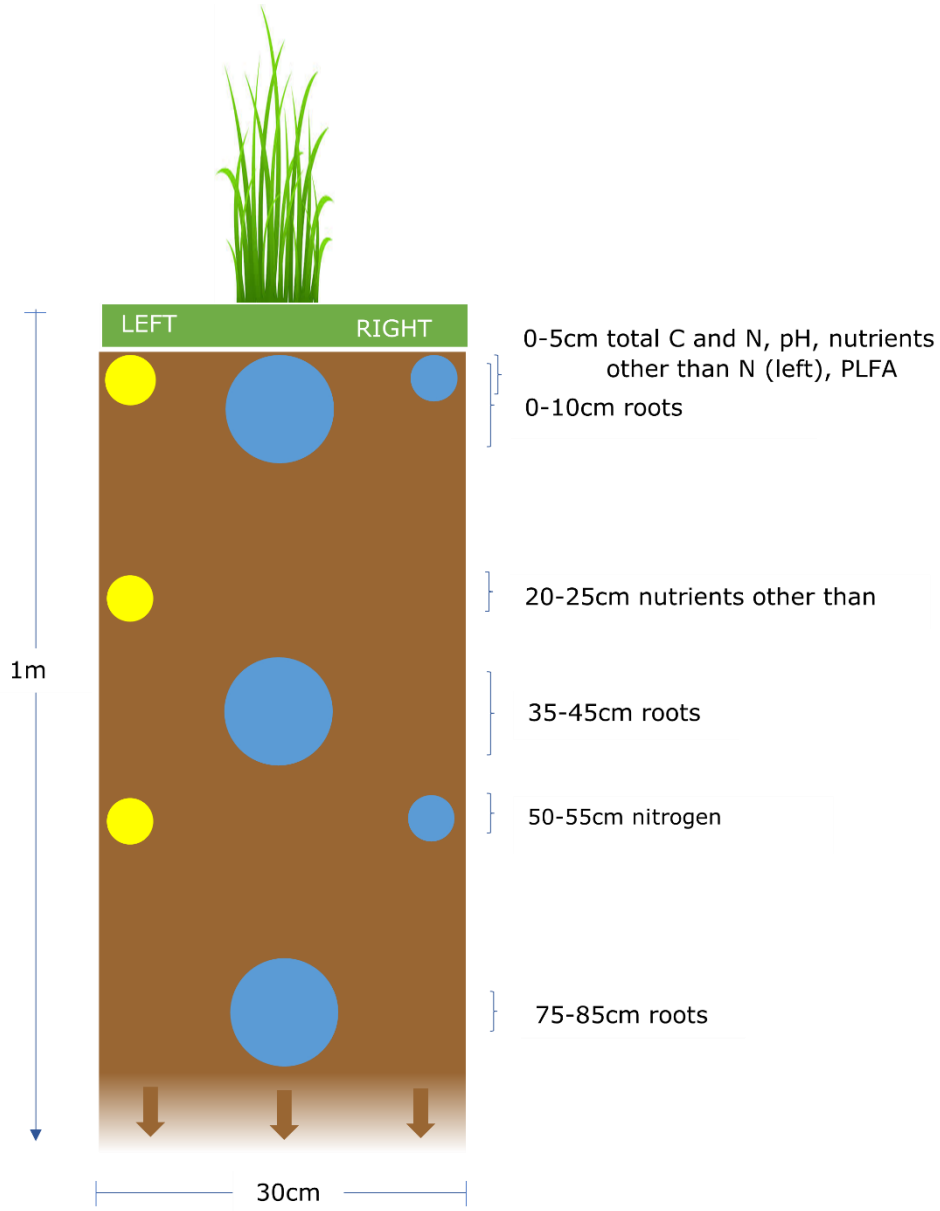

Fig. 1 Rhizotron and soil sampling design. Yellow highlighted samples were taken 69 days after mulch application. Samples to estimate nutrients other than N (P, K, Mg, Fe, Mn, Zn, and Cu) were included in these. Blue highlighted samples were taken on day 195 after mulch application. Large blue circles at the middle indicate root extraction samples

Barley seeds, barley plants and soil samples for root extraction from the depths of 0-10, 35-45, and 75-85 cm were also collected during this timepoint. Roots were extracted by submerging soil samples into

tap water over a 1 mm sieve and collecting the floating roots. All plant materials were dried at 80°C for 48 to 72 hours to constant weight.

Soil and plant material analysis

Measurements were conducted on plant residues, on barley plants and on soil samples to estimate physical and chemical properties.

Total Carbon, Nitrogen and protein of seeds and plant residues, C:N ratio of plant residues, as well as total Carbon and Nitrogen of soil samples were estimated with combustion by LECO CHN 628 analyser (LECO Corporation). Dry mass of plant residues and barley plants was estimated by drying at 80°C for 48 to 72 hours to constant weight and of soil samples at 105°C for 24 hours. ANKON 2000 Fibre Analyser (ANKON Technology) was used to measure recalcitrant substances of plant residues according to ANKOM Technology protocols (ANKON Technology, 2020). Samples were first dried at 80°C to constant weight and grinded to < 1mm in a Fritsch grinder by Glen Creston Ltd. Plant residue fiber analysis was consisted of 3 successive processes to determine the % Neutral Detergent Fiber (NDF) (Hemicellulose, Cellulose and Lignin), the % Acid Detergent Fiber (ADF) (Cellulose and Lignin), and the % Acid Detergent Lignin (ADL) (Lignin). The % Cellulose was estimated by subtracted the % ADL from the % ADF, and the % Hemicellulose by subtracting the % ADF from the % NDF. Neutral Detergent Solution (NDS), Alpha-amylase and anhydrous sodium sulfite were used as reagents for % NDF, Acid Detergent Solution for % ADF, and Sulfuric acid 72% by weight for % ADL.

Soil solution pH was measured with a calibrated pH meter. Air dry soil samples of 10 g, sieved to < 2 mm, were put in centrifuge tubes where 25 mL of ultra-pure water were added, and the tubes were shaken for 15 minutes on an end over end shaker before measurement (Blakemore, Searle, & Daly, 1987). Available N in soil samples was estimated by KCl extraction, according to standard protocol (Great Britain. M.A.F.F., 1986), colorimetrically in a San Continuous Flow Injection Analyzer (SKALAR Instruments) using 40 g of air-dry soil in 200 ml 1M potassium chloride solution. The concentration of atoms of the available P, K, Mg, Mn, Fe, Cu and Zn nutrients for plant uptake in soil samples were evaluated with Mehlich 3 method by Perkin Elmer – Optima 7300 DV ICP-OES analyser (AgroEcoLab UMD; PerkinElmer, Inc.) using air dry soil samples of 2 g and 20 ml of Mehlich 3 extracting solution (Mehlich, 1984; Pierzynski, 2000).

Soil microbial biomass of gram+ bacteria, gram- bacteria, fungi, protozoa, and cyanobacteria as well as the fungi: bacteria ratio in freeze dry soil samples of 3 ±0.1 g were estimated with Phospholipid Fatty Acid (PLFA) analysis as described by Sizmur et. al (2011). Samples were analysed by an Agilent 6890N Network GC System Gas Chromatographer (Agilent Technologies). Methyl tetradecanoate (C14:0) internal standard, Methyl nonadecanoate C19:0 Internal standard, Bacterial Acid Methyl Ester (BAME) Mix Quantitative standard, and Supelco 37 Component (FAME) Mix Quantitative standard were used. The chromatograms were elaborated by Agilent ChemStation – G2190BA – B04.03 software (Agilent

ChemStation company). The Retention Time Window was set to 0.10 min and 1.5%, with Methyl tetradecanoate (C14:0) and Methyl nonadecanoate (C19:0) internal standards as Reference compounds. Soil samples were freeze dry in an Edwards Super Modulyo freeze dryer (Edwards company) soon after their collection and stored to -20^{0}C until the analysis.

Two air dried and sieved to <2mm soil samples were used to estimate soil texture with a hydrometer by adding 50 ml sodium hexametaphosphate solution (SHMP) at 50 g/l to 40 g soil subsamples (Bouyoucos, 1962). Soil water holding capacity (WHC) was estimated gravimetrically using 50 g fresh soil samples with the method described by Harding and Ross (1964). The height of the main stem of barley plants from the soil surface to the base of the flag leaf was used as a proxy of growth rate (cm/d).

Statistical analysis:

All statistical analyses were carried out in Minitab 19 (Minitab, LLC) except Principal Component Analysis which was conducted in R-Studio (RStudio, PBC). All measurements from a treatment were averaged to obtain a single mean value, the unit of replication of this study being the rhizotron (n=5), and $\alpha = 0.05$ was used to denote significance. One-way ANOVA was used to detect differences between treatments or residue types. Measurements repeated in time (day 69, and day 195) or soil depth were analyzed by Mixed Effects Model with rhizotrons as random factor and treatments or residue type, time, and soil depth as fixed factors. All data subjected to analyses of variance were tested for normality and homogeneity using Darling-Anderson and Levene tests. When these conditions were not satisfied, Kruskal-Wallis test was conducted in the case of one-way ANOVA, while in the other cases transformation of data with $\log_{10}$ or Box-Cox transformation with optimal or rounded λ was made. Where there were significant differences a Tukey post-hoc test was conducted. Comparisons with control treatment were made using the Dunnett test. Data was separated and analyzed at a specific sampling time or depth, where normality or equality of variances were not satisfied even after data transformation. Descriptive statistics included means and standard deviations rather than standard errors unless it was otherwise stated.

Regression analysis was conducted to examine the significance and the degree of the effect of a single independent variable to a response variable in cases where there was an apparent influence (residue dry mass loss vs initial residue NDF or N content, soil K vs initial residue C:N ratio). Homogeneity of variance of the data was tested with Levene test, and normality of data distribution with Anderson-Darling test. Spearman and Pearson correlations were used to assess the significant correlations between variables. The Spearman correlation was also used when normality or equality of variances of data in regression analysis were not satisfied.

Multivariate analysis was also conducted using Principal Component Analyses (PCAs) to assess differences between treatments in several variables simultaneously, and the relations of the

treatments with those variables concerning measurements on soil nutrients or on soil microbial biomass or on barley plants.

Results

Residue dry mass loss and initial residue quality

The dry mass loss was significantly different between residues of different diversity (types) (N = 5, F = 72.14, p-value < 0.001). $S_{(6)}$ type had the highest dry mass loss, while *W* had the lowest, and there were no significant differences between $P_{(1)}$, $B_{(12)}$, and $H_{(17)}$ types (Fig. 2, and Table S2).

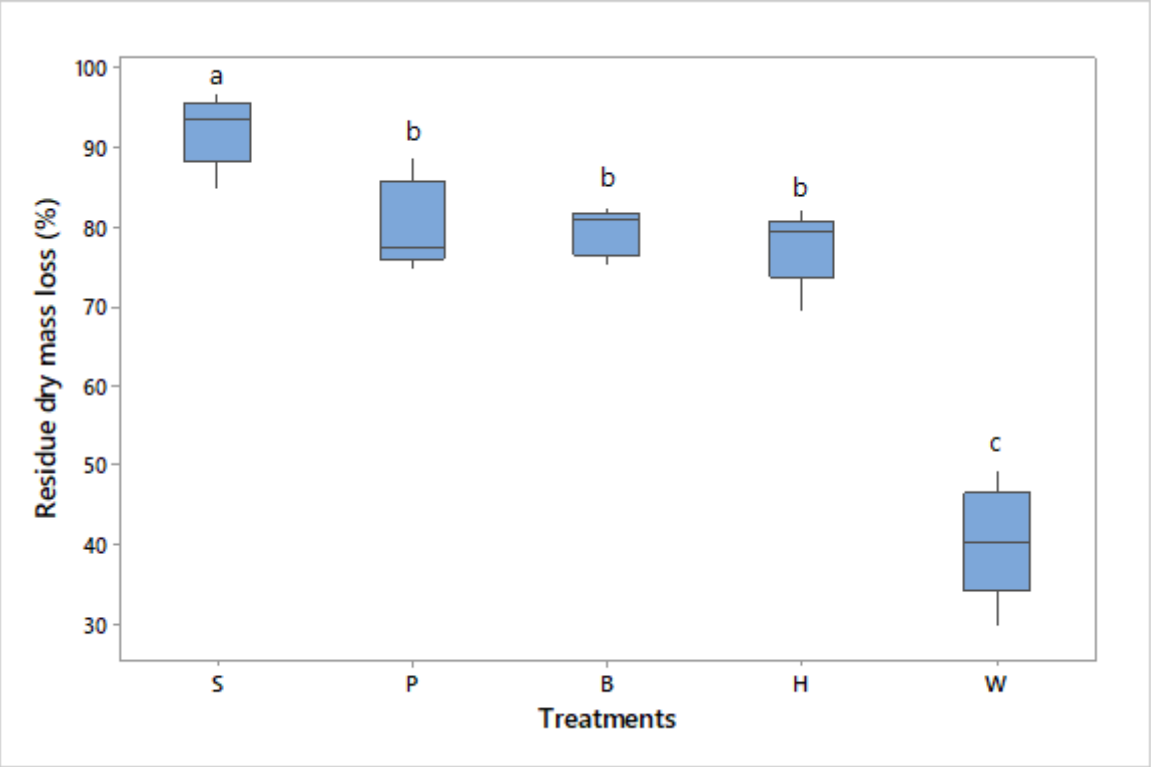

Fig. 2 Residue % dry mass loss of the different treatments at the end of the growing season (day 195 after mulch application) (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means that do not share a letter are significantly different (p<0.05)

Table 1 Classification of residue types in descending order of their initial or final mean values according to different properties. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin). Means that do not share a letter are significantly different (p<0.05)

Properties	Residue Types	Properties	Residue Types
Initial C:N ratio	$W^a > H^b > S^c > B^c > P^d$	% initial NDF	$W^a > H^b > B^{bc} > P^c > S^d$

Initial % C	$W^a > P^b > H^c > S^c > B^d$	% initial ADF	$W^a > B^b > H^c > S^d > P^d$
Initial % N	$P^a > S^b > B^c > H^d > W^e$	% initial ADL	$W^a > B^a > H^b > P^b > S^b$
Species richness	$H > B > S > W > P$	Dry mass loss	$S^a > P^b > B^b > H^b > W^c$

The initial C:N ratio was significantly different between residues of different diversity (N = 6, F = 12143.32, p-value < 0.001, $\log_{10}$ transformation of data). All types were significantly different between them except $S_{(6)}$ and $B_{(12)}$ (Fig. S3, and Tables 1, S3, and S4). Kruskal-Wallis test showed significant differences in initial C of residues between diverse residues (H-value = 26.69, p-value < 0.001). Although normality of data was not satisfied, Tukey test showed significant differences between all types except between $H_{(17)}$ and $S_{(6)}$ (Fig. S3, and Tables S3, and S5). The initial N between diverse residues was significantly different (N = 6, F = 10509.87, p-value < 0.001). All types were significantly different between them (Fig. S3, and Tables S3, and S6).

Pearson correlation matrix (Table S7, N = 5) between initial C:N ratio, C, and N content of residues showed significant negative correlation between C and N (r = -0.533, p = 0.002) considering all residue types together. Values of correlation index r between C and N, considering each type alone, showed significant negative correlation for type $H_{(17)}$, and significant positive correlation for type $B_{(12)}$.

The initial hemicellulose was significantly different between residue types (N = 5, F = 109.48, p-value < 0.001). All types were significantly different between them except $P_{(1)}$ and $H_{(17)}$ which had the highest values (Tables S8, and S9). The initial NDF content of residues was significantly different between residue types (Kruskal-Wallis test, N = 5, H-value = 22.46, p-value < 0.001). Although normality of data was not satisfied, Tukey test indicated significant differences between all types except between $H_{(17)}$ and $B_{(12)}$ and between $B_{(12)}$ and $P_{(1)}$. *W* type had the highest value and $S_{(6)}$ the lowest (Fig. S4, Tables S8, and S9). The initial cellulose was significantly different between residue types (N = 5, F = 41.46, p-value < 0.001). *W* type which had the highest value was significantly different from all other types, while there was nearly significant difference between $H_{(17)}$ and $P_{(1)}$ types (Tables S8, and S10). The initial ADF content of residues was significantly different between all types except between $S_{(6)}$ and $P_{(1)}$ types which had the lowest values (F = 1743.45, p-value < 0.05, N = 5, Fig. S4, and Tables S8, and S10). The initial ADL (lignin) content of residues was significantly different between residue types (N = 5, F = 10.33, p-value < 0.001). *W* and $B_{(12)}$ types had the highest values not significantly different between them, while all other types with lower values were also not significantly different between them (Fig. S4, and Tables S8, and S11). NDF content was significantly correlated with both ADF and ADL, and the same was true also between ADF and ADL (Table S12). Moreover, residue dry mass loss was significantly negatively correlated with NDF, with ADF, with lignin, and with cellulose, but not with hemicellulose, considering all treatments together (Table S12). Regression analysis showed that initial residue NDF could significantly (N = 5, F = 196.31, p-value < 0.001) explain r^2 = 89.51 % of residue dry mass loss variability. The regression equation was: residue dry mass loss = 165.01 - 2.073 NDF (Fig. S5).

Moreover, initial residue N content could significantly (N = 5, F = 34.29, p-value < 0.001) explain 59.85 % of the residue mass loss, with the quadratic plot as the best fitted (N = 5, r^2 = 88.7 %, F = 55.87, p-value < 0.001, Fig. S6), with regression equation: residue dry mass loss = -5.016 +55.76 N – 8.534 N^2.

Effect on soil solution pH and on C and N mineralization

Soil solution pH, measured at 0-5 cm depth, was not significantly different between treatments in samples taken on day 69 or on day 195 (Table S13). In both timepoints Tukey's post-hoc test showed that soil solution pH was significantly lower in the bulk soil (initial soil, prior to its use in rhizotrons) (6.36 ±0.0346) than in any other treatment (N = 5, F = 68.33, p-value < 0.001 for day 69, and F = 61.59, p-value < 0.001 for day 195). Moreover, the pH was significantly (N = 30, F = 50.96, p-value < 0.001) increased on day 195 (7.7193 ±0.1519) than on day 69 (7.4597 ±0.1171). Spearman correlation showed a statistically significant negative correlation (N = 5, ρ = -0.426, p-value = 0.034) between soil solution pH and total soil microbial biomass on day 69.

At 0-5 cm depth, total soil C was not significantly different between treatments or between sampling timepoints (day 69 and day 195). The same was true for total soil N between day 69 and day 195, but it was significantly lower in *Control* (0.15626 ±0.01078) than in $P_{(1)}$ (0.17203 ±0.01332) treatment, considering both day 69 and day 195 together (N = 10, T-value = 3.12, p-value = 0.034). On day 69 the bulk soil had significantly higher total soil N (0.18519 ±0.01071) than the $S_{(6)}$ treatment (0.15743 ±0.01197) (N = 5, T-value = -3.44, p-value = 0.027) (Tables S14, and S15). On day 195, bulk soil had significantly higher value in total soil N than *Control* (which had the lowest value), *W*, and $H_{(17)}$ treatments. *Control* had significantly different value also from $S_{(6)}$, $B_{(12)}$, and $P_{(1)}$ treatments (Tables S14, and S15).

Both on day 69 and on day 195, available N was detected only as NH_4^+ and not as NO_2^- and NO_3^- in samples from 50-55 cm depth. NH_4^+ were not significantly different between treatments in either timepoint, but they were significantly higher on day 69 (2.489 ±0.621) than on day 195 (1.4993 ±0.305) considering all treatments together (N = 30, T-value = -8.32, p-value < 0.001). There were significant differences between treatments in interaction with time (Table S16). All treatments had significantly lower values than the bulk soil (2.765 ±0.385) in NH_4^+ content only on day 195 (N = 5, Kruskal-Wallis test, H-value = 19.49, p-value = 0.003).

Effect on soil nutrients other than nitrogen

Soil content of nutrients P, K, Mg, Fe, Mn, Zn, and Cu was estimated by samples taken from 0-5 and 20-25 cm depth on day 69. All elements had significant differences in values between the two depths, except P (Table S17). K was the only element where significant differences had been detected between treatments at combined samples from those depths (N = 10, F = 3.74, p-value = 0.012). More specifically, soil K in *Control* (62.19 ±14.03) was significantly lower than in $H_{(17)}$ (78.65 ±21.24, T-value

= 3.97, p-value = 0.007) and in $P_{(1)}$ (75.78 ±15.06, T-value = 3.28, p-value = 0.033) treatments (Fig. S7). Although there were no significant differences between treatments in the other elements, some interesting observations could be made. *W* and *Control* treatments had the lowest values, and $H_{(17)}$ treatment the highest in soil P. *Control* had the lowest value and *W* the highest in soil Mg. *Control* had the lowest value in soil Fe, and $H_{(17)}$ the highest. $H_{(17)}$ had the highest value in soil Mn, and *Control* of the lowest. $S_{(6)}$ treatment followed by $H_{(17)}$ had the highest soil Zn value, while *W* had the lowest. *W* had the highest value in soil Cu, followed by $H_{(17)}$ treatment.

Further investigation on soil K was made for comparisons of *Control* with the other treatments at each depth separately. At 0-5 cm depth soil K in *Control* was significantly lower than from all other treatments (N = 5, F = 3.65, p-value = 0.013), while at 20-25 cm depth soil K in *Control* was significantly lower only with $H_{(17)}$ treatment (N = 5, F = 2.27, p-value = 0.08, T-value = 3.07, adjusted p-value = 0.022) (Table S18, Fig. 3). Although $H_{(17)}$ was the treatment with the highest initial C:N ratio except *W* treatment, regression analysis did not show any significant influence of initial residue C:N ratio on soil K on day 69 at 20-25 cm depth, either including *W* treatment (N = 5, F = 1.96, p-value = 0.175) or not (N = 5, F = 0.11, p-value = 0.747).

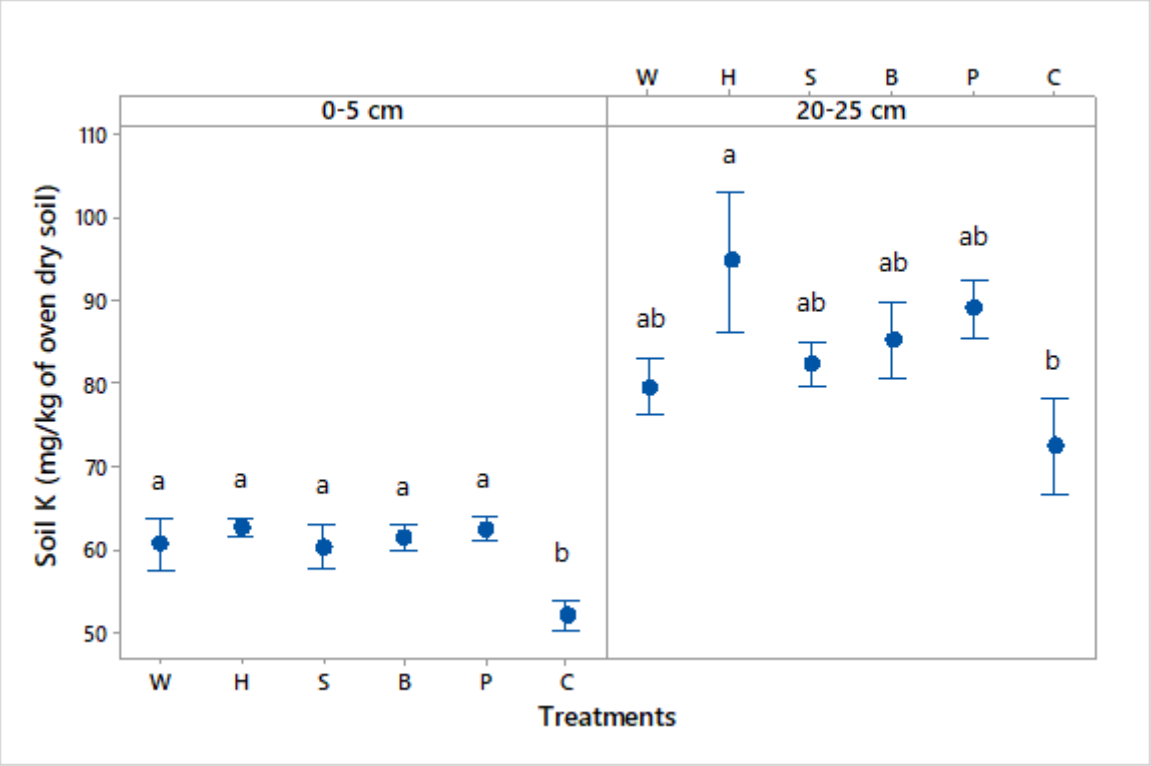

Fig. 3 Soil K content (mg/kg of oven dry soil) for the different treatments (*B, C, H, P, S,* and *W*), on day 69 after mulch application at 0-5 and 20-25 cm depths (N = 5). Means and bars of one standard error from the mean are depicted. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. *Control (C)* treatment was with no residues. Treatments that do not share a common letter are significantly different (p-value < 0.05)

Considering all samples from both 0-5 and 20-25 cm depth in Dunnett test's comparisons, bulk soil (N = 4) had significantly higher values in both soil K, and soil Mg than any treatment (N = 10), and significantly lower value than $H_{(17)}$ treatment in soil Fe (T-value = 2.88, adjusted p-value = 0.022) (Table S19). Generally, bulk soil had higher values in macronutrients K, and Mg, and lower in micronutrients Fe, Zn, and Cu.

Principal component analysis was conducted to detect differences between treatments considering all soil nutrients simultaneously. Variables included soil nutrients P, K, Mg, Fe, Mn, Zn, and Cu (Fig. S8). In Fig. S8a at 0-5 cm depth, biplot shows that treatments were overlapped. However, *Control* was negatively related with all soil nutrients, mainly with Fe, Mn, and Zn, while $P_{(1)}$ treatment was negatively related with P. $H_{(17)}$ and $S_{(6)}$ treatments were positively related with Fe, Mn, and Zn. *W* treatment was positively related with Mg, and Cu, while $B_{(12)}$ treatment seemingly was not related with any nutrient.

In Fig. S8b at 20-25 cm depth, all treatments were overlapped. However, $H_{(17)}$ treatment was positively related with K, Mn, and Fe. $P_{(1)}$ treatment was positively related with K. *W* treatment was negatively related with P, Mg, Fe, Mn, Zn, and Cu. *Control* was negatively related mainly with K, while $B_{(12)}$, and $S_{(6)}$ treatments apparently were not related with any nutrient.

Effect on soil microbes

A number of 24 compounds were considered in Phospholipid Fatty Acid analyses on soil samples from 0-5 cm depth from both day 69 and day 195 timepoints to evaluate decomposer microbes' groups (nmol g^{-1} of freeze dry soil). The groups were represented as follows: Gram+ bacteria by C14:0, iC15:0, a-C15:0, C15:0, i-C16:0, i-C17:0, C18:0, and C20:0, Gram- bacteria by 2-OH-C12:0, C14:1, C15:1, 2-OH-C14:0, and 3-OH-C14:0, Fungi by C16:0, C18:3ω6, C18:2ω6c, C18:2ω6t, C18:1ω9c, C18:3ω3, and C20:1ω9, Protozoa by C20:4ω6 and C20:3ω6, and Cyanobacteria by C20:5ω3, although in previous studies C18:2ω6c has also been detected in Cyanobacteria, and C18:1ω9c and C18:3ω3 in higher plants and green algae (Quideau et al., 2016; Buyer & Sasser, 2012; Amir et al., 2010; Zelles, 1999; Frostegård & Bååth, 1996; Vestal & White, 1989).

On day 69, $S_{(6)}$ treatment had the highest value than all the other treatments in all microbial groups, but significantly different only in Cyanobacteria (N = 5, Kruskal-Wallis test, H-value = 11.75, p-value = 0.038). It is remarkable that treatments followed the same pattern of PLFA values $S_{(6)}>P_{(1)}>B_{(12)}>H_{(17)}>W>C$ in descending order for all microbial groups with slight alterations. In protozoa $B_{(12)}$ treatment displayed higher value than $P_{(1)}$, and *Control* higher than *W* treatment. In Gram+, and in Gram- groups *W* treatment had higher value than $H_{(17)}$ treatment. The order $S_{(6)}>P_{(1)}>B_{(12)}>H_{(17)}>W$ was exactly the same with that of dry mass loss and reversed of that of recalcitrance. Moreover, the values between $B_{(12)}$ and $P_{(1)}$ and between $H_{(17)}$, *W,* and *C* were quite close (Tables S20, and Fig. 4).

On day 195, there were no significant differences between treatments, in all cases of microbial groups. However, the values in $S_{(6)}$ treatment were clearly lower than those on day 69. The tendency in PLFAs between treatments for each microbial group was reversed from that of day 69. The pattern of PLFA values in descending order was $S_{(6)}>H_{(17)}>W>C>P_{(1)}>B_{(12)}$ with small alternations between $H_{(17)}$ and $S_{(6)}$, or $H_{(17)}$ and W and between $P_{(1)}$ and C, or $P_{(1)}$ and $B_{(12)}$. The values between $S_{(6)}$, $H_{(17)}$, and W and between C, $P_{(1)}$, and $B_{(12)}$ were quite close (Table S21, and Fig. 4).

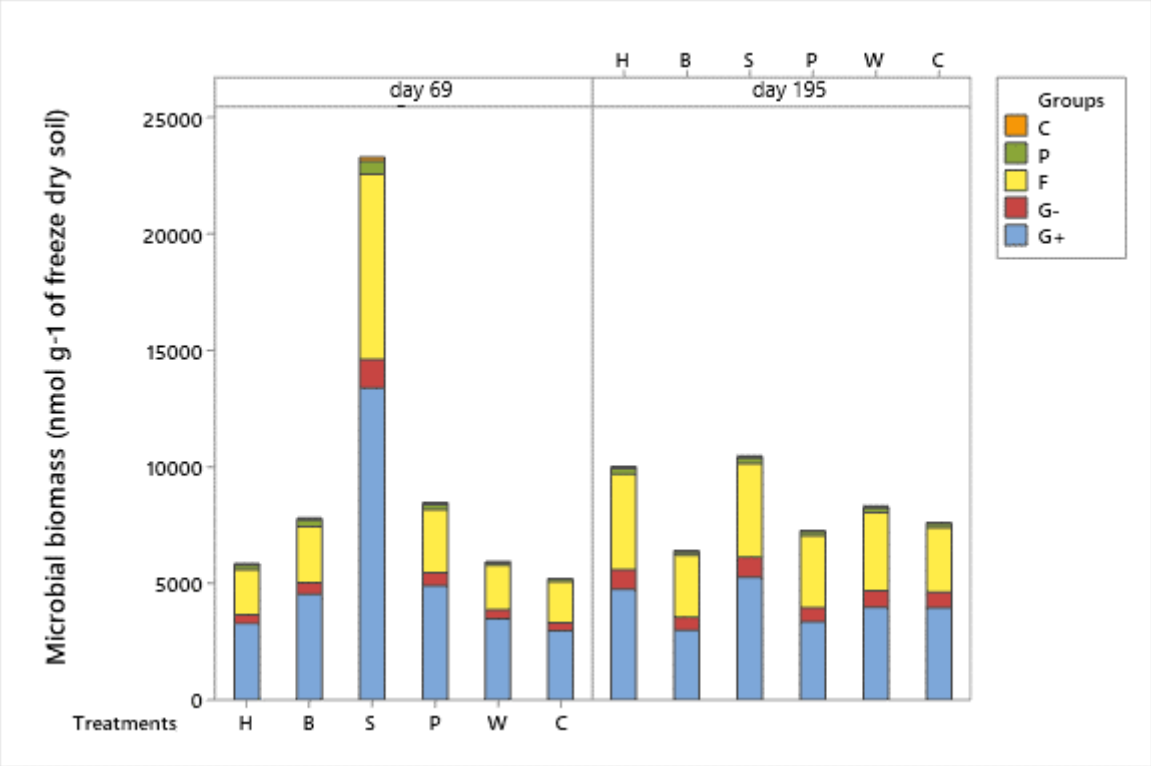

Fig. 4 Mass of the different microbial groups (G+ = gram positive bacteria, G- = gram negative bacteria, F = fungi, P = protozoa, and C = Cyanobacteria), expressed in nmol g^{-1} of freeze dry soil, for the different treatments (*H, B, S, P, W,* and *Control (C)*) from soil samples at 0-5 cm depth, on day 69 and on day 195 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*

The total microbial biomass according to the selected compounds was not significantly different between treatments, both on day 69 (N = 5, Kruskal-Wallis test, H-value = 5.34, p-value = 0.376) and on day 195 (N = 5, F-value = 0.89, p-value = 0.501). The differences in descending order were $S_{(6)}>P_{(1)}>B_{(12)}>W>H_{(17)}>C$ on day 69, and $S_{(6)}>H_{(17)}>W>C>P_{(1)}>B_{(12)}$ on day 195 (Table S22). Considering all treatments together, the microbial biomass was not found significantly different between day 69 (1882 ±2712) and day 195 (1667 ±748), but further investigation is needed because normality of data was not satisfied even after transformation. Spearman correlation showed a statistically significant positive correlation (N = 5, ρ = 0.432, p-value = 0.031) between total microbial biomass on days 69 and 195, but there were no significant correlations (N = 5, p-values > 0.05) on each day (69 and 195) between

microbial biomass and initial residue chemical characteristics (C:N ratio, C, N NDF, ADF, and lignin content).

The differences between treatments in soil microbial groups were also tested with Principal Component Analyses, for day 69 (Fig. 5) and for day 195 (Fig. 6).

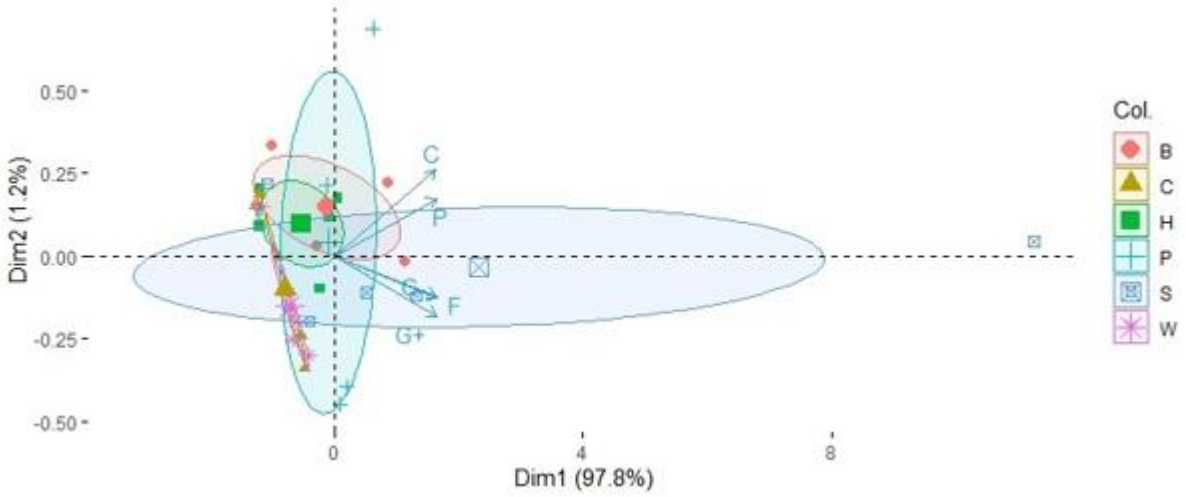

Fig. 5 Biplot of PCA for the different treatments (*B, Control (C), H, P, S,* and *W*) with microbial groups (G+ = Gram positive bacteria, G- = Gram negative bacteria, F = fungi, P = protozoa, and C = Cyanobacteria) as variables. Soil samples were collected from 0-5 cm depth, on day 69 after mulch application. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Large points depict mean values, while ellipses depict confidence intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1 normalized score, and left axis represents Dim2 normalized score

Biplot of PCA in Fig. 5 shows that variables were related in 2 major groups on day 69: C and P formed one, and F, G+ and G- another. *Control* and *W* treatment were plotted similarly and distinctly different from $B_{(12)}$ and $H_{(17)}$ treatments. *Control* and *W* were negatively related with all microbial groups but mainly with C and P, while $B_{(12)}$ and $H_{(17)}$ were also negatively related with all microbial groups but mainly with F, G+, and G- groups. Clearly, $S_{(6)}$ treatment was positively related with G+, G-, and F groups, while $P_{(1)}$ treatment apparently was not particularly related with any microbial group.

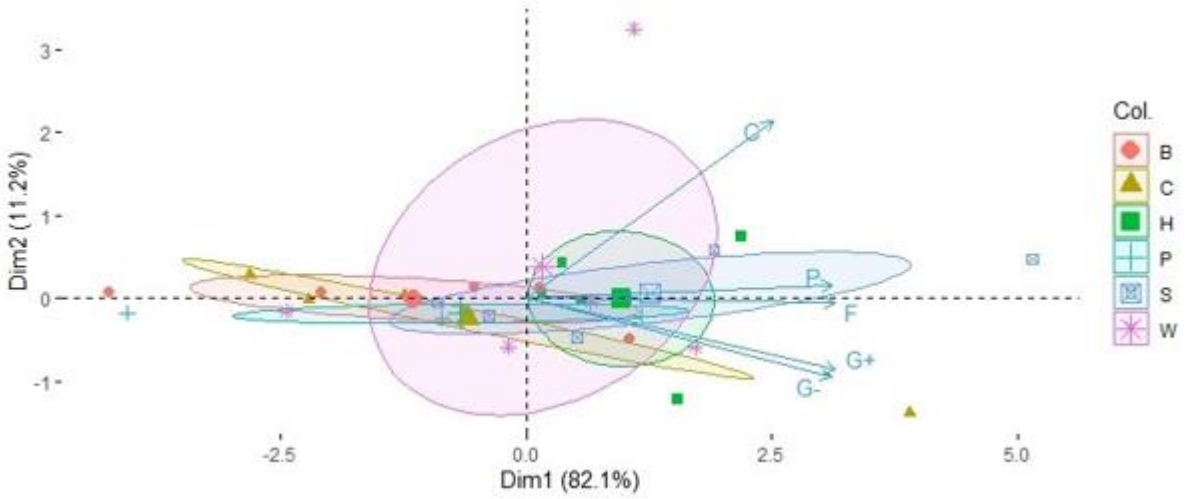

Fig. 6 Biplot of PCA for the different treatments (*B, Control (C), H, P, S,* and *W*) with microbial groups (G+ = Gram positive bacteria, G- = Gram negative bacteria, F = fungi, P = protozoa, and C = Cyanobacteria) as variables. Soil samples were collected from 20-25 cm depth, on day 195 after mulch application. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Large points depict mean values, while ellipses depict confidence intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1 normalized score, and left axis represents Dim2 normalized score

Biplot of PCA in Fig. 6 shows that variables were related in 3 groups on day 195: C the first group, P and F the second, and G+ and G- the third. Although all treatments were overlapped, $S_{(6)}$, and $H_{(17)}$ treatments were positively related mainly with both P and F, but also with G+ and G- microbial groups. *W* treatment was positively related with Cyanobacteria. $P_{(1)}$ and $B_{(12)}$ treatments were negatively related mainly with F and P groups. *Control* was negatively related mainly with G+ and G- groups. PCA is a visualized depiction of relations between values and variables, and it should be interpreted in combination with Tables S22 and S23.

The fungi: bacteria ratio on day 69 was 0.515, taken Gram+ and Gram- as total bacteria biomass (nmol of compounds per g of freeze dry soil), and considering all treatments together, while the mean values of treatments in descending order was $S_{(6)}>H_{(17)}>C>P_{(1)}>W>B_{(12)}$. On day 195, the fungi: bacteria ratio was raised to 0.708 and the order of the mean values of treatments was $P_{(1)}>B_{(12)}>H_{(17)}>W>S_{(6)}>C$, while the difference was slight in *Control* treatment (Fig. 4, and Table S24).

Effect on barley plants

Barley root dry mass estimated from soil samples at 75-85 cm depth was not significantly different between treatments (N = 5, Kruskal-Wallis test, H-value = 5.19, p-value = 0.394). $H_{(17)}$ treatment (0.0463 ±0.0361) had the highest value followed by *Control*, and $B_{(12)}$ (0.02006 ±0.01686) had the lowest. At 35-45 cm depth also there were no significant differences of root dry mass between

treatments (N = 5, F = 1.02, p-value = 0.427). *Control* had the highest value (0.0462 ±0.0246), followed by $H_{(17)}$ treatment (0.04250 ±0.01649), and again $B_{(12)}$ had the lowest (0.03012 ±0.00814). Contrary, there were significant differences of root dry mass between treatments at 0-10 cm depth (N = 5, F = 2.82, p-value = 0.038). $H_{(17)}$ treatment had the highest value (0.5448 ±0.1168), significantly different from *W* which had the lowest (0.3305 ±0.0884) (T-value = -3.23, p-value = 0.037, Table S25).

The $H_{(17)}$ treatment had the highest value (33.61 ±3.13) of barley plant dry mass (g) (without seeds and roots), followed by $P_{(1)}$, $B_{(12)}$, and $S_{(6)}$ in descending order, while *Control* (28.75 ±2.66) and *W* (27.16 ±6.94) had the lowest, but the differences were not statistically significant (N = 5, F = 0.92, p-value = 0.488).

Yield as expressed by seeds' dry mass per plant (g) was not significantly different between treatments (N = 5, F = 1.13, p-value = 0.374). $H_{(17)}$ treatment had the highest value (31.22 ±2.60) followed by *W* (25.27 ±9.33), while *Control* had the lowest (22.85 ±3.50). *W* treatment gave the heaviest seeds (4.927 ±0.324) followed by $H_{(17)}$, *C,* $P_{(1)}$, $B_{(12)}$, and $S_{(6)}$ in descending order, although not significantly different (N = 5, F = 1.63, p-value = 0.189). The number of ears per plant and the number of tillers per plant as other criteria to estimate crop yield, also showed no significant differences between treatments (N = 5, F = 1.33, p-value = 0.285, and F = 1.17, p-value = 0.351, respectively). $H_{(17)}$ had both the highest number of ears per plant followed by $B_{(12)}$, $S_{(6)}$, $P_{(1)}$, *W,* and *Control*, and the highest number of tillers per plant followed by $P_{(1)}$, $B_{(12)}$, $S_{(6)}$, *W*, and C in descending order.

Protein content of seeds was not significantly different between treatments (N = 5, F = 0.32, p-value = 0.898). However, there were significant differences between treatments in C content of seeds (N = 5, F = 3.64, p-value = 0.014). $B_{(12)}$ treatment (44.568 ±0.133) had significantly higher value than *Control* (44.157 ±0.232, T-value = -3.50, p-value = 0.020) and $H_{(17)}$ (44.192 ±0.247, T-value = -3.20, p-value = 0.039) (Table S26).

Main stem height (cm) of barley plants seems to follow a double S curve at all treatments from day 32 to day 116 after plant emergence (Fig. S9) (Boyle, 2017). Total main stem elongation rate (cm/d) of the different treatments between each timepoint and day 32 after plant emergence, growing stage GS31 (Tottman et al., 1986), is depicted in Fig. 7.

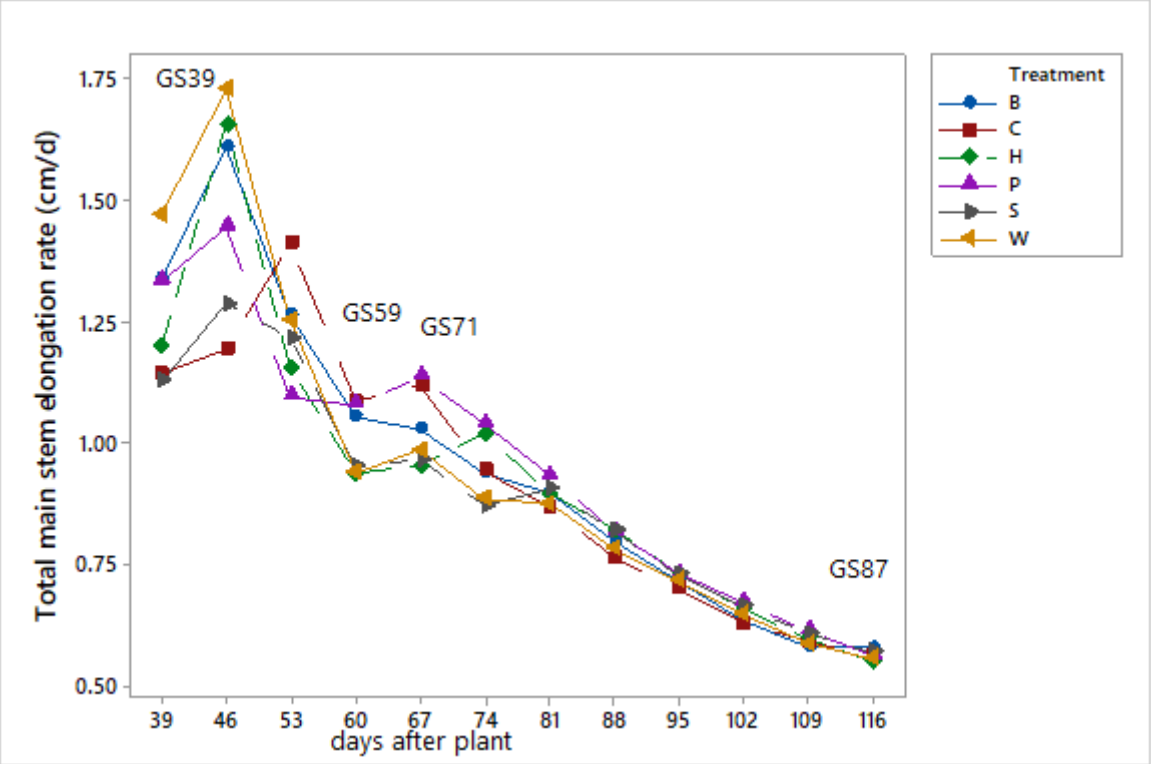

Fig. 7 Total main stem elongation rate (cm/d) of the different treatments (*B, Control (C), H, P, S,* and *W*) measured from 26th July to 1st November 2019 (rates among each timepoint and the 26th of July). Values derived from damaged stems were omitted and the next highest stem was considered instead. GS = Barley growing stage (Tottman et al., 1986). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*

Total growth rate, represented by the total main stem elongation rate, was initially rising, reaching its peak 46 day after plant emergence (GS39) and steadily declining thereafter. *Control* reached its peak in main stem elongation rate a little later than the rest of the treatments (on day 53 after plant emergence). No significant differences on both main stem height and total stem elongation rate have been found between treatments at each timepoint. Spearman correlation was significantly negative between yield and seed protein content (ρ = -0.709, p-value < 0.001), and between seed C and initial residue C:N ratio (ρ = -0.441, p-value = 0.027), and significantly positive between seed C and seed protein content (ρ = 0.417, p-value = 0.038). In contrast there were no significant Spearman correlations between main stem elongation rate (at any time point) and yield or seed protein or seed C or initial residue chemical composition (C:N ratio, C, N, NDF content) (p-values > 0.05).

Principal Component Analysis (PCA) was conducted to detect differences between treatments considering many variables simultaneously. The included variables were: v1 = total barley dry mass (g), v2 = root dry mass (g) in soil samples from 0-10, 35-45, and 75-85 cm depth, v3 = final number of tillers, v4 = yield (seed dry mass per plant), v5 = % seed protein, and v6 = soil NH_4^+ (mg/kg oven dry soil) at harvest time (Fig. 8).

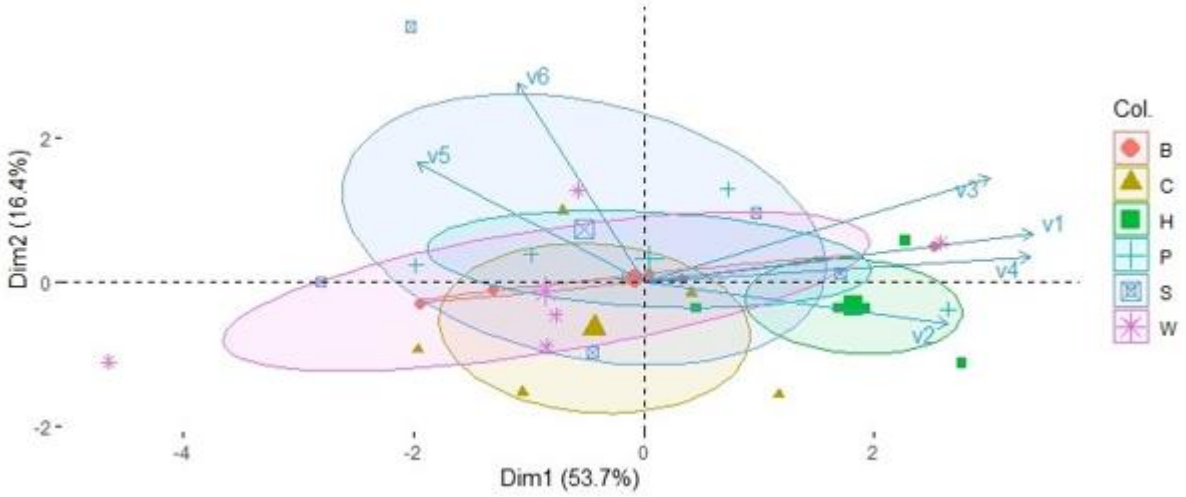

Fig. 8 PCA biplot of variables v1 = total barley dry mass (g), v2 = root dry mass (g) in soil samples from 0-10, 35-45, and 75-85 cm depth, v3 = final number of tillers, v4 = yield (seed dry mass per plant), v5 = % seed protein, and v6 = soil NH_4^+ (mg/kg oven dry soil) on day 195 after mulch application, for the treatments *B, Control (C), H, P, S,* and *W*. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Large points depict mean values, while ellipses depict confidence intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1 normalized score, and left axis represents Dim2 normalized score

Fig. 8 shows that all treatments were overlapped but $H_{(17)}$ treatment was distinctly different than all the others. $H_{(17)}$ was clearly positively related with barley root dry mass (v2) more than any other treatment, and $S_{(6)}$ treatment was positively related with seed protein and soil NH_4^+ content (v5 and v6). *W* treatment and *Control* were negatively related with total barley dry mass, final number of tillers, and yield (v1, v3, and v4). $B_{(12)}$ treatment apparently was not related with any variable.

Discussion

Previous research has shown that direct N mineralization is usually expected when C:N ratio of the applied residues for decomposition is equal or less than 24, because the C:N ratio of the body of soil decomposer microorganisms is around 8 and they assimilate about 1:3 of the C released (Brust, 2019). However, this threshold of residue C:N ratio can be higher when soil microbes and fauna with a C:N ratio of their body more than 8 are involved in decomposition (Frouz *et al.*, 2015). According to Vigil and Kissel (1991) the break-even point of residue C:N ratio between N mineralization and immobilization can be as high as 40. Therefore, in our experiment direct net N mineralization was expected in all treatments with residues (C:N ratio ranged from 10.7 to 16.8), except *W* type (C:N ratio 50.1) where initial N immobilization was expected. Initial C and N content of residues considering all types together was significantly negatively correlated but considering each type alone both significant

positive and negative correlations were observed (Table S7). This possibly is due to inhomogeneity of the sampling material, which constitute an inevitable limitation in experiments with residue mixtures.

NDF was considered as a measure of comparison for recalcitrance because it was significantly positively correlated with both ADF and lignin and in addition it includes all recalcitrant fractions (hemicellulose, cellulose, and lignin). Both hemicellulose and cellulose are characterized by intermediate and lignin by slow decomposition rate (Hadas *et al.*, 2004). The higher the NDF the higher the recalcitrance. Indeed, residue dry mass loss had significant negative correlation with NDF, and also with cellulose and lignin, but not with hemicellulose. This was in agreement with a study where lignin and cellulose, but not hemicellulose, were related with net N mineralization of plant residues (Zhonglu *et al.*, 2015). Also, Jensen et al. (2005) reported that holocellulose (hemicellulose and cellulose together) could explain more effectively the C mineralization rate from above-ground residues than lignin. Therefore, all three factors (NDF, N, and C) must be considered in residue dry mass loss investigation rather than C:N ratio alone. In previous experiments, usual factors that have been used to explain differences in dry mass loss were residue C:N ratio, N:P ratio, lignin, cellulose, or polyphenol content of residues and combinations of them (Xu *et al.*, 2017; Zhonglu *et al.*, 2015; Vahdat *et al.,* 2011). The significant influence of initial residue NDF and N content in residue mass loss was confirmed by regression analysis. It has been shown that NDF had significant linear negative correlation with mass loss, while N content had positive correlation up to a threshold which turned to be negative after that value as it was shown by the quadratic plot. This was in line with previous research which showed that residue decomposition can be reduced by the addition of N due to microbial competition (Fog, 1988), and it seems to be true over a threshold of residue N content in relation with other factors such as the residue NDF content.

$S_{(6)}$ treatment which had high quality residues with the lowest recalcitrance resulted in the highest residue dry mass loss, 56.35 % higher than that of *W* treatment (Table 1). In addition, the fact that $S_{(6)}$ treatment had clearly the highest soil microbial biomass than any other treatment, both on day 69 and on day 195 (Table S22), indicates the relationship between soil microbial biomass and residue mass loss. It has been shown that plant litter with low C:N ratio causes increased respiration activity (Tahir and Marschner, 2017; Swift et al., 1979). However, the considerably higher microbial biomass in $S_{(6)}$ treatment in comparison to the other treatments, mainly on day 69, cannot be explained merely by its high-quality residue. A possible explanation may be an increased development of herb weeds under the mulch, favoured by increased soil N availability in $S_{(6)}$ treatment, and the consequent increased microbial biomass at their rhizosphere. Nevertheless, it is obvious (Table S22) that high quality residues, resulted in higher activity of microbial biomass on day 69, and consequently higher residue dry mass loss than low quality residues. Probably these conditions offered by high quality residues ensured adequacy in available N and other nutrients for microbial decomposers at that time, in

comparison to treatments with lower quality residues (Tahir and Marschner, 2017; Campbell *et al.*, 1993).

Reversely, residues of lower quality ($H_{(17)}$, and *W* treatments) showed enhanced microbial biomass on day 195 in comparison to residues of higher quality but they still resulted in lower mass loss, because the time period of this reversed trend was shorter, and in addition decomposition was decelerated over time (Ostrofsky, 2007). Residues of lower quality initially drove to lower N availability, and in reduced activity for soil microbial decomposers. These conditions influenced decomposition at least until day 69 and the influence was reversed sometime up to day 195. These results are an indication that residues of higher quality were initially decomposed faster than those of lower quality, but the decomposition rate was reversed at later stage, as we hypothesised. They also show that differences in decomposition rate was accompanied by analogues differences in soil microbial activity. However, our hypothesis was not fully confirmed because, in all cases, differences were not significant. In addition, Spearman correlations failed to reveal any significant influence of initial residue chemical characteristics on soil microbial biomass at each of the days 69 and 195, but they revealed an influence of soil microbial activity to lower the soil solution pH on day 69. In addition, Spearman correlations failed to reveal any significant influence of initial residue chemical characteristics on soil microbial biomass at each of the days 69 and 195, but they revealed an influence of soil microbial activity to lower the soil solution pH on day 69, in agreement with previous research (Singh & S. Khind, 1992; Fog, 1988). This observed influence of microbial activity on soil solution pH on day 69 but not on day 195 is probably attributed to deceleration of decomposition rate over time.

Interestingly, *W* treatment (C:N ratio > 40) did not have significant difference in microbial biomass than any other treatment on both day 69 and day 195, although it demonstrated significantly lower dry mass loss than the other treatments (Tables 1 and 2). It seems that microbial activity is not always strongly correlated with microbial biomass. Shu et al. (2021) found greater soil microbial respiration at residues of higher species richness in comparison to the single constituent plants, attributed to activation of dormant microbes, but not significant differences in soil microbial biomass. However, the fact that *Control* treatment resulted in the lowest microbial biomass on day 69 and quite low on day 195, highlights the contribution of plant residue mulch in the abundance of soil microbes at advanced stages of decomposition. Besides, the difference in microbial biomass between Control and the other treatments was expected to be reduced in accordance with previous research (Shu et al., 2021). Lower soil diversity in *Control* in terms of quantity and structure implies soil abiotic deterioration conditions in comparison to the mulch treatments (Tibbett *et al.*, 2019), which was confirmed in results on soil nutrient contents. The lack of significant differences between treatments in soil microbial biomass at advanced stages had been also notified by Bremer et al. (1991) who observed evolution of similar amounts of CO_2 by incubation of both high- and low-quality plant residues in soil, although the CO_2 evolution was initially faster from the high-quality residues. Nevertheless, the lack of significant

differences on total microbial biomass between day 69 and day 195, considering all microbial groups and treatments together, although mean values were higher on day 69, is an indication that decomposition rate was decelerated over time, but microbial activity was not reduced on day 195 and that was true for all treatments.

Table 2 Classification of treatments (*B, Control (C), H, P, S,* and *W*) in descending order, according to their values in microbial biomass for the different microbial groups as well as in total microbial biomass on day 69 and on day 195 after mulch application. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. The * denotes significant differences between treatments (N = 5, Kruskal-Wallis test, H-value = 11.75, p-value = 0.038)

Microbial groups	Day 69	Day 195	Total on day 69	Total on day 195
Gram+	*S>P>B>W>H>C*	*S>H>W>C>P>B*	*S>P>B>W>H>C*	*S>H>W>C>P>B*
Gram-	*S>P>B>W>H>C*	*S>H>W>C>P>B*		
Fungi	*S>P>B>H>W>C*	*H>S>W>P>C>B*		
Protozoa	*S>B>P>H>C>W*	*S>H>W>P>C>B*		
Cyanobacteria	*S>P>B>H>W>C**	*S>W>H>C>B>P*		

It has been stated that soil microbial diversity is governed by temporal, spatial, and hierarchical variability, and that aboveground nutrient fluxes may affect belowground diversity (Lukac, 2017). Indeed, a decrease in Gram+, Protozoa, and Cyanobacteria biomass was observed in residues of higher quality ($B_{(12)}$, $P_{(1)}$, and $S_{(6)}$) from day 69 to day 195, and an increase in residues of lower quality (*W*, and $H_{(17)}$) but also in Control (Table S23). This denotes that in high quality residues these three microbial groups are favoured at early stages of decomposition, while in residues of lower quality they remain active for longer period and their biomass still increases at later stages of decomposition. Therefore, the progress of this early stage is slower and prolonged in residues of lower quality. Moreover, Gram- bacteria and fungi were increased in residues of lower quality in much higher rates from day 69 to day 195 than in residues of higher quality. These findings are in line with previous research (Liang *et al*., 2017; Nottingham *et al*., 2009). Probably fungi were favoured by increased recalcitrance on day 195, and both fungi and Gram- bacteria were benefited by feeding on the increased bacterial necromass resulted by microbes that decomposed the easily decomposable material at earlier stage (Nottingham *et al*., 2009). Moreover, Gram- bacteria were more competitive as they were favoured by relatively higher moisture content on soil surface (Chen *et al.*, 2020) under residues of lower quality which remained more intact with longer particle size due to slower decomposition rate in comparison to residues of higher quality.

The fungi: bacteria ratio, considering Gram+ and Gram- as bacterial biomass, on day 69 was lower than on day 195 for all treatments, while the difference was slight in *Control* (Table S24). As decomposition

proceeded, its rate was decelerated, and residue recalcitrance was increased (Xu et al., 2017). Lower recalcitrance on day 69 favoured bacteria, while fungi were favoured by increase of recalcitrance on day 195 (Liang et al., 2017; Grosso *et al.,* 2016). Bacteria would be expected to take over again in a long term, after the first year, at both high or low quality residues, as it was shown in a three-year experiment (Frasier et al., 2016). In our experiment, fungi: bacteria ratio was not significantly different between residues of higher or lower quality on both day 69 and day 195. Logically, low quality residues would be expected to result in higher fungi: bacteria ratio at least on day 195. However, this ratio remained higher in residues of higher quality than in lower quality residues, although fungal biomass was much more increased in residues of lower quality, because the biomass of the Gram- bacteria was increased even more (Table S23, and S24). This is justifiable because in previous research, it has been shown that low quality residues increase both the ratios of fungi: bacteria and Gram-negative: Gram-positive bacteria (Liang et al., 2017). Moreover, lower dry mass loss and increased microbial biomass on day 195 by residues of lower quality implies a higher fertilization capacity at later stage of decomposition by them in comparison to high quality residues in accordance with our hypothesis. However, this hypothesis was not fully confirmed because differences in microbial biomass were not significant. The results from PCA for PLFA analysis reinforced the assumption of higher microbial activity in residues of higher quality than those of lower quality on day 69 and the opposite on day 195. The observed negative relations of *Control* with microbial groups on both day 69 and day 195 (late stages of decomposition) was in agree with previous research (Calderón *et al.,* 2016).

The effect of residue diversity and quality on soil microbial populations were well reflected in soil chemistry. All treatments, except $S_{(6)}$, maintained total soil N content close to that of the bulk soil (prior to its use in the experiment) on day 69. This shows that mineralized and immobilized N compensated the amount taken up by barley plants. It is possible the development of ephemeral herbaceous weeds under residues in $S_{(6)}$ treatment led to lower values of total soil N in this treatment due to increased plant nutrient uptake (Table S14). On day 195, treatments with high-quality residues ($S_{(6)}$, $P_{(1)}$, and $B_{(12)}$) still maintained total soil N content close to that of the bulk soil. Contrary, treatments with residues of lower quality ($H_{(17)}$, and *W*) had significantly lower values and *Control* had the lowest. This shows a positive contribution of mulch to soil N dynamics even on day 195, which was higher in residues of higher quality, although soil microbial biomass was increased in residues of lower quality. It is also possible that N became the limiting factor in low-quality residues due to increased microbial activity. Higher soil N has also been observed in treatments with higher quality forage mixtures, compared to lower quality, by other researchers using the same types of mixtures ($H_{(17)}$, $B_{(12)}$, $S_{(6)}$, $P_{(1)}$) but only in a wet than in a drier soil (Shepperd *et al.*, 2020). However, the precedency of high-quality residues on total soil N was not reflected on soil NH_4^+ content at 50-55 cm depth (Table S27). This is another indication of slow deposition of nutrients from long size plant-based mulch, which drive to avoidance of N loss by leaching, in contrast to homogeneous incorporation of residues into soil (Ambus *et al.,*

2001), although mulching could increase N losses in the form of NH_3 by volatilization (Singh & S. Khind, 1992).

Cultivation of barley resulted in decrease of soil macronutrients K and Mg, and in increase of micronutrients Fe, Zn, and Cu than bulk soil, significantly different in all cases, except Zn, considering soil samples from both 0-5 and 20-25 cm depths, on day 69. The release of nutrients is decelerating over time and may not compensate plant uptake at later stages of decomposition (Reichert *et al.*, 2015). Moreover, *Control* treatment demonstrated significantly lower soil K than any other treatment, the lowest Mg, and Fe, and of the lowest P, and Mn, although not significantly different. This is an indication that all treatments with mulch contributed to enrichment of soil with essential nutrients, mainly macronutrients but also with micronutrients, which is in line with other research (Wang *et al.*, 2021). Soil macronutrient content was significantly higher (K, and Mg) or nearly significantly higher (P) at 20-25 than at 0-5 cm depth, probably due to leaching (Table S17). Inorganic P is more prone to soil fixation at 0-20 cm depth, while organic forms are more prone to leaching (Khan et al., 2018). Plant residue decomposition constitutes a significant source of K, which is released as ion to soil solution and like Mg it is easily subjected to leaching (Xie *et al.*, 2020). Soil micronutrient Fe, Mn, and Cu content was significantly higher at 0-5 cm depth, while Zn content was significantly higher at 20-25 cm depth (Table S17). Concentration of micronutrients are normally higher at the surface soil layer and is decreasing with depth (Fageria et al., 2002).

K was the only nutrient, apart from N, where significant differences have been found between treatments (Fig. S7, and Table S18). Soil K was higher in residues with higher C:N ratio, but not too much higher. *W* treatment with C:N ratio >40 contributed little on enrichment of soil K, although its contribution was higher than the *Control*. However, simple regression analysis failed to reveal any significant influence of initial residue C:N ratio alone to soil K. It seems, residues of higher quality released higher amounts of K earlier due to higher initial rates of decomposition, while the opposite was true with lower quality residues (Campbell *et al.*, 1993). $H_{(17)}$ treatment had the highest mean value in K, P, Fe, and Mn, and of the highest in Zn, Cu, and Mg, considering both depths 0-5 and 20-25 cm (Tables S28, and S29). Clearly, it was the treatment with the higher contribution in enrichment of soil with nutrients on day 69. This possibly may be attributed to the higher species richness of $H_{(17)}$ in comparison to the other treatments, which triggered the activation of more diverse soil microbial population resulting in mineralization of higher amounts of nutrients (Santonja et al., 2017). Diverse residue mixtures have been proposed by researchers as an alternative to crop diversity to provide nutritional benefits in cases where monocultures cannot be avoided (Struijk *et al.*, 2020). Moreover, mulch of mixtures of different recalcitrance has been found to increase soil macronutrient content (Wang *et al.*, 2021). PCA biplots for the nutrients (Fig. S8a, and S8b) confirmed negative relations of *Control*, and *W* treatments with most nutrients, possibly due to lack of N availability. It also confirmed positive relations of $H_{(17)}$ with most nutrients.

Sampling time to detect soil nutrients was at barley plants' GS59 to GS60, when emergence of inflorescence was completed for most of the plants and the flowering was beginning. At this stage, although the growth of leaves had been ceased, stems were still growing, and ear growth was accelerated. Green area production had been largely completed and nutrients started to be used mainly for seed development (Boyle, 2017). As it has already been shown treatments of high-quality residues had the highest values of total soil N at 0-5 cm depth on both day 69, and day 195, significantly different from *Control*. This was reflected in higher values of protein, N, and C content of seeds at harvest time than the other treatments. Seed protein content is increased with increasing N supply (Awopegba *et al.*, 2017) and specifically in barley seeds when enough N is available after flag leaf emergence or no earlier than GS32 (Boyle, 2017). Contrary, $H_{(17)}$ treatment was highly positively related with barley plant biomass, both overground and root biomass as well as with yield and negatively related with protein content of barley seeds, as it is evident by PCA of barley plants. *W* treatment and *Control* had low barley plant biomass, low yield, and low seed protein content, probably connected with low N availability. Apart from considerable higher C:N ratio, *W* had significantly higher lignin content than $H_{(17)}$ treatment. Lignin is considered to restrict N mineralization rates because it degrades to phenolic compounds which interact with plant proteins and amino acids forming resistant to decay humic polymers (Fox et al., 1990). The results related to crop yield, to barley plant biomass, to seed protein, and to seed N content were not significantly different between treatments and must be interpreted as an indication of influence. However, seed C content of $B_{(12)}$ treatment was significantly higher than Control and $H_{(17)}$ treatment confirming our hypothesis that residue quality affects the quality of crop production but not crop yield. It is possible, continuous application of whole-plant residue mulch will lead to more profound significant differences in crop yield and quality, but this assumption must be proved.

According to their influence on barley plants, and considering PCA results, treatments could be classified in three categories. In first category belong treatments characterized by low initial residue C:N ratio, high residue N content, and low recalcitrance ($B_{(12)}$, $P_{(1)}$, and $S_{(6)}$ treatments), resulting in high seed protein content, and high barley plant biomass, but low yield. In second category are treatments characterized by low initial residue C:N ratio, low residue N content, and high recalcitrance ($H_{(17)}$ treatment), resulting in low seed protein content, high barley plant biomass, and high yield. In third category are treatments characterized by high initial residue C:N ratio, low residue N content, and high recalcitrance (*W* treatment), resulting in low seed protein content, low barley plant biomass, and low yield. *Control* treatment (without residues) have similar response with those in the third category.

Treatments *W*, $H_{(17)}$, and $B_{(12)}$ had similar behaviour in main stem elongation rate, displaying the highest mean values at growing stage GS39 where rapid stem elongation occurs. These treatments were those with the highest recalcitrance than all the other. This is an indication of possible influence of residue functional traits on nutrient plant uptake and consequently on nutrient dynamics and crop quality.

Moreover, *Control* showed a delay in reaching its peak in main stem elongation rate, in comparison to the other treatments. This was reflected to lower mean value of yield and is an indication of the beneficial effect of long size mulch on plant nutrition and yield. It has been shown in a previous research that plant-based mulch contributed to increase of seed protein content and crop yield in comparison to no mulch treatment (Awopegba et al., 2017). However, Spearman correlation did not confirm any influence of main stem elongation rate either with crop quality and yield or with initial residue chemical composition. Nevertheless, it confirmed significant correlation of residue functional traits with crop quality and yield because initial residue C:N ratio was negatively correlated with seed C which was positively correlated with seed protein which was negatively correlated with yield. The fact that differences between treatments on crop quality (in most cases) and on yield in our experiment were not significant denotes that any influence of whole-plant mulch on crop yield and quality was slow. Regular mulch application, and adaptation of appropriate field management systems are necessary in order the long-term benefits to become apparent. However, the experiment was in rhizotrons, therefore, results should be considered with caution and be tested in real field conditions.

Conclusions

Mulch of high-quality long residues (30 cm) resulted in higher mass loss than residues of lower quality, 195 days after mulch application (day 195). Residue mass loss was significantly negatively correlated with initial residue NDF and N content. Soil microbial biomass was higher in residues of lower quality on day 195, opposite of day 69, in consistent patterns, which shows a reverse trend in decomposition rate between residues of higher and lower quality, although differences were not significant. This finding, in combination with the results on residue mass loss, implies that residues of lower quality possibly maintained higher capacity as fertilizers on day 195 than residues of higher quality, but our hypothesis was not fully confirmed because differences on microbial biomass were not significant. At the same time there were no significant differences between treatments in crop yield. Soil fungal: bacterial ratio was increased with the progress of decomposition, although not significantly, for all treatments with residues, indicating an increase in residue recalcitrance, while the difference was negligible in *Control*. Residues of higher quality resulted in increased total soil N, both on day 69 and on day 195 than residues of lower quality and *Control*, but differences were significant only between residues of higher quality and *Control* on day 195, while there were no significant differences between treatments in soil NH_4^+ content on both days. This shows that mulch could continuously supply soil with N, even at advanced stages of decomposition, avoiding at the same time N losses by leaching. Soil K was significantly increased in treatments with residues in comparison to *Control* on day 69. Residues of higher quality resulted in significantly higher seed C content than residues of lower quality and *Control*. The same was true for seed protein content, although not significantly, but a significant positive correlation between seed C and seed protein content was found. These results show that crop quality is more readily affected by residue functional traits than crop yield, confirming our hypothesis.

Generally, long residue diverse mulch can contribute to enrichment of soil nutrients, may affect seed crop quality, and possibly may affect soil microbial population at the later stages of the cultivation period. However, any influence of residue diversity or quality on nutrient dynamics, microbial biomass, seed quality, and crop yield is slow and not always detectable. Further research is needed with continuous application of diverse long-residue mulch in no tillage or conservation tillage practices to investigate its effect on chemical and biological soil properties, on crop yield, and on crop quality.

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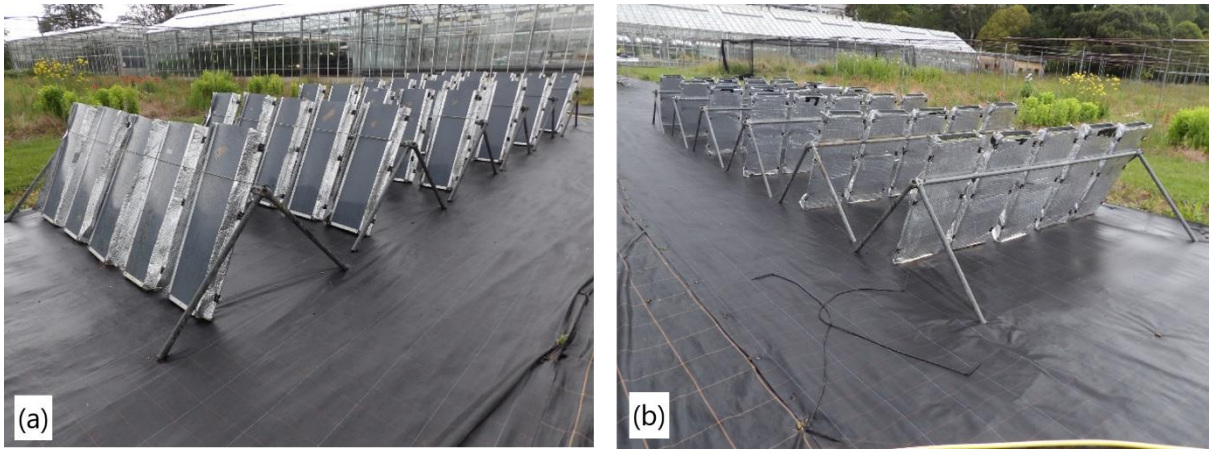


Fig. S1 Rhizotrons set at the outdoor. From left to right a) back side, b) front side with the glass covered with alluminum foil

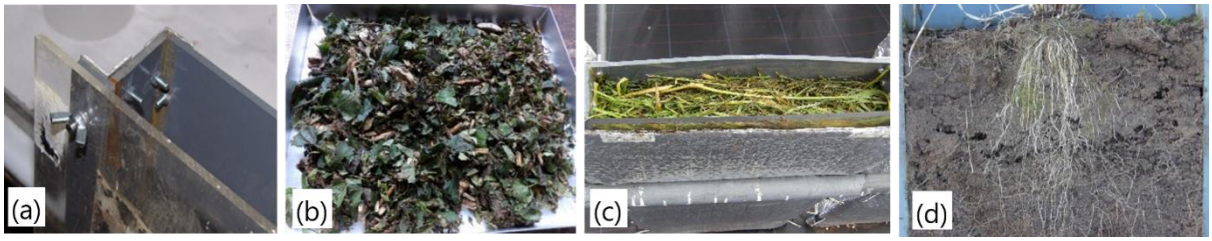
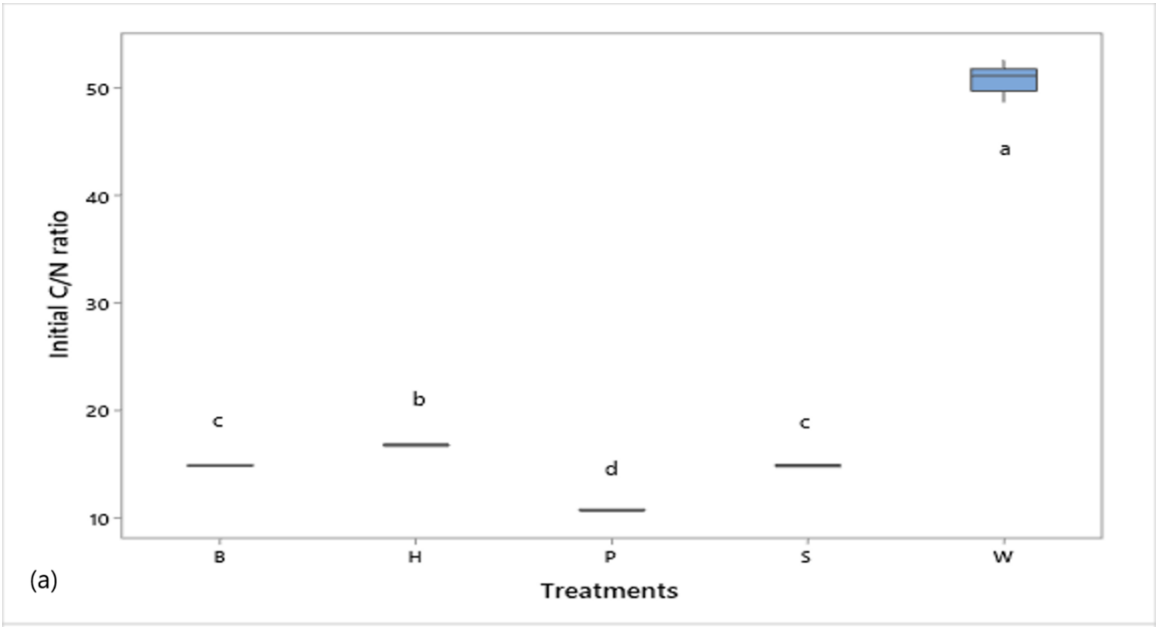


Fig. S2 From left to right a) easily removable plexy glass on rhizotrons, b) wood chips (shredded branches and twigs with their leaves), c) mulch application, d) root development at the glass side

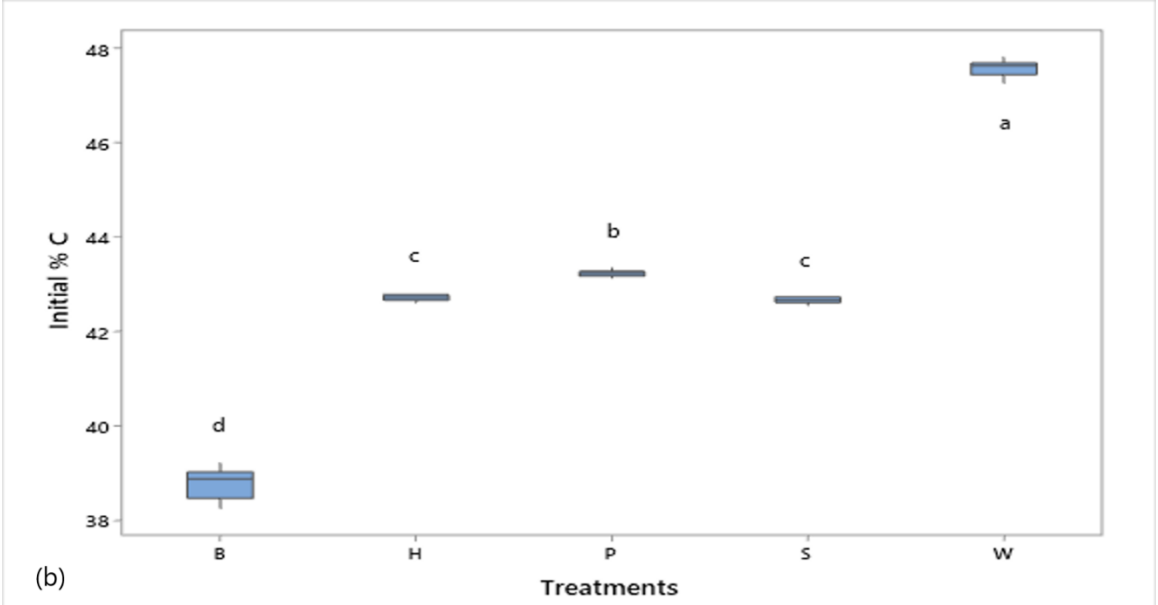
Table S1 Diverse forage mixture species selection list (*P*: Perennial Ryegrass; *S*: Smart Grass; *B*: Biomix; *H*: Herbal)

Species	**Latin**	***P***	***S***	***B***	***H***
Perennial Ryegrass	*Lolium perenne* L.	✓	✓	✓	✓
Timothy	*Phleum pratense* L.		✓	✓	✓
Cocksfoot	*Dactylis glomerata* L.			✓	✓
Festulolium	-			✓	✓
Tall Fescue	*Festuca arundinacea* Schreb.				✓
Meadow Fescue	*Festuca pratensis* Huds.			✓	✓
Red Clover	*Trifolium pratense* L.		✓	✓	✓
White Clover	*Trifolium repens* L.		✓	✓	✓
Alsike Clover	*Trifolium hybridum* L.			✓	✓
Sweet Clover	*Melilotus* spp.				✓
Black Medick	*Medicago lupulina* L.			✓	

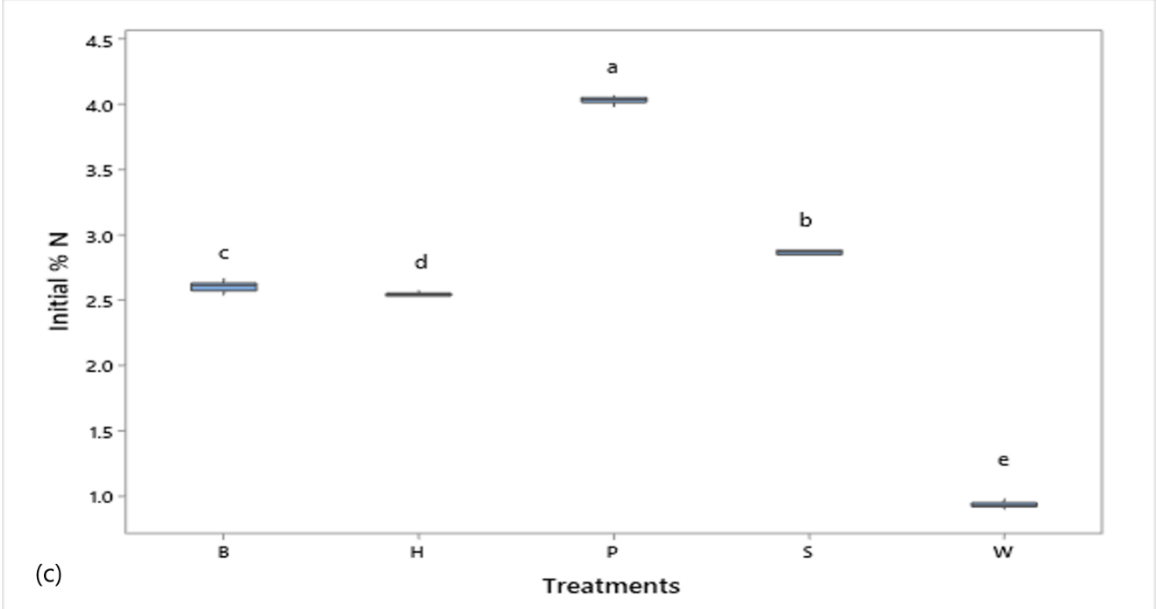
Lucerne	*Medicago sativa* L.		✓	
Sainfoin	*Onobrychis spp.*			✓
Birdsfoot Trefoil	*Lotus corniculatus* L.			✓
Plantain	*Plantago lanceolata* L.	✓	✓	✓
Chicory	*Cichorium intybus* L.	✓	✓	✓
Yarrow	*Achillea millefolium* L.			✓
Burnet	*Sanguisorba minor* Scop.			✓
Sheep's Parsley	*Petroselenium crispum* Mill.			✓


Initial C/N ratio
50
40
30
20
10
c
b
d
c
a
B
H
P
S
W
Treatments

(a)


Initial % C
48
46
44
42
40
38
d
c
b
c
a
B
H
P
S
W
Treatments

(b)


Initial % N
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
c
d
a
b
e
B
H
P
S
W
Treatments

(c)

Fig. S3 Initial (a) C:N ratio (b) % C, and (c) % N content of the residues of the different treatments (*B, H, P, S,* and *W*) (N = 6). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means that do not share a letter are significantly different (p<0.05)

Table S2 Dry mass loss (%) of the different residue types, and Tukey's post-hoc test for differences between residue types (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means that do not share a letter are significantly different, and p-values <0.05 indicate significant differences

Treatment	Mean ±StDev	Difference of Levels	T-Value	Adjusted P-Value	Difference of Levels	T-Value	Adjusted P-Value
B	79.42 ±2.94[b]	*H - B*	-0.56	0.980	*S - H*	4.42	0.002
H	77.60 ±4.78[b]	*P - B*	0.21	1.000	*W - H*	-11.41	<0.001
P	80.11 ±5.52[b]	*S - B*	3.87	0.008	*S - P*	3.66	0.012
S	92.09 ±4.48[a]	*W - B*	-11.97	<0.001	*W - P*	-12.18	<0.001
W	40.20 ±7.23[c]	*P - H*	0.76	0.938	*W - S*	-15.84	<0.001

Table S3 Initial C:N ratio, % C, and % N content of residues the different treatments (*B, H, P, S,* and *W*) (N = 6). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means that do not share a letter are significantly different (p<0.05)

	C/N	% C	% N
Treatments	Mean ±StDev	Mean ±StDev	Mean ±StDev
B	14.873 ±0.106[c]	38.794 ±0.336[d]	2.6086 ±0.0405[c]
H	16.780 ±0.118[b]	42.720 ±0.0641[c]	2.5460 ±0.0148[d]
P	10.719 ±0.0722[d]	43.238 ±0.0700[b]	4.0338 ±0.0269[a]
S	14.877 ±0.106[c]	42.667 ±0.0704[c]	2.8681 ±0.0186[b]
W	50.865 ±1.321[a]	47.579 ±0.179[a]	0.93589 ±0.02385[e]

Table S4 Tukey's post-hoc test for differences between treatments (*B, H, P, S,* and *W*) in initial C:N ratio of residues (N = 6). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

Difference of Levels	T-Value	Adjusted P-Value	Difference of Levels	T-Value	Adjusted P-Value
H - B	15.76	<0.001	*S - H*	-15.72	<0.001
P - B	-42.77	<0.001	*W - H*	144.80	<0.001
S - B	0.04	1.000	*S - P*	42.80	<0.001
W - B	160.56	<0.001	*W - P*	203.32	<0.001
P - H	-58.52	<0.001	*W - S*	160.52	<0.001

Table S5 Tukey's post-hoc test for differences between treatments (*B, H, P, S*, and *W*) in initial % C of residues (N = 6). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

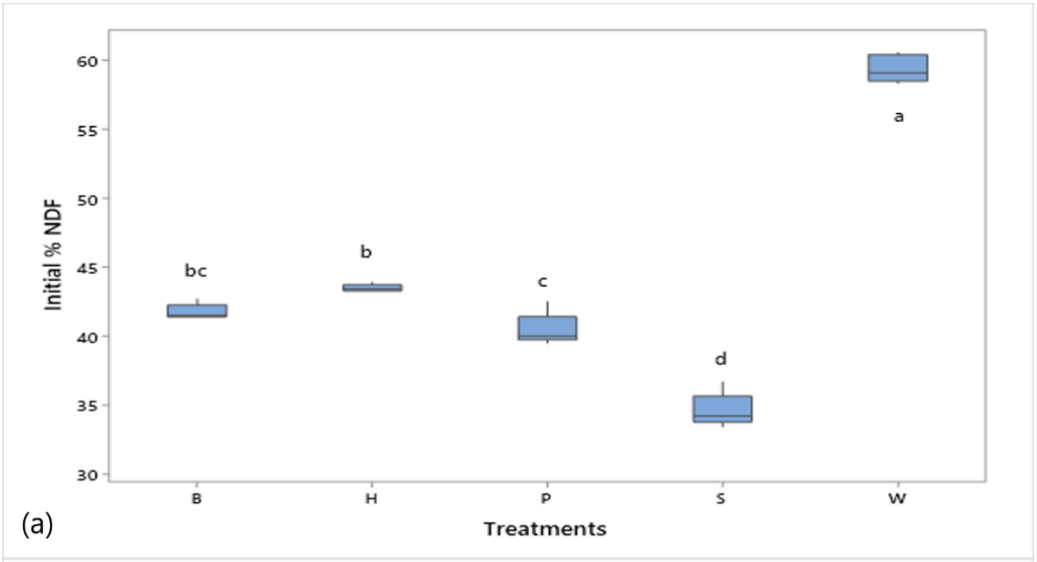
Difference of Levels	T-Value	Adjusted P-Value	Difference of Levels	T-Value	Adjusted P-Value
H - B	38.15	<0.001	*S - H*	-0.51	0.985
P - B	43.18	<0.001	*W - H*	47.22	<0.001
S - B	37.64	<0.001	*S - P*	-5.54	<0.001
W - B	85.37	<0.001	*W - P*	42.19	<0.001
P - H	5.03	<0.001	*W - S*	47.73	<0.001

Table S6 Tukey's post-hoc test for differences between treatments (*B, H, P, S,* and *W*) in initial % N ratio of residues (N = 6). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

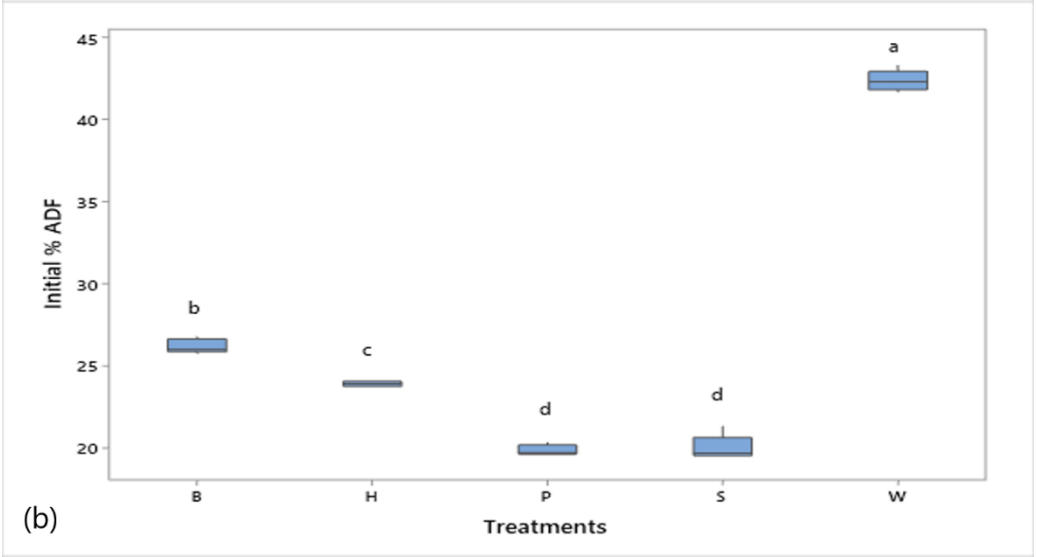
Difference of Levels	T-Value	Adjusted P-Value	Difference of Levels	T-Value	Adjusted P-Value
H - B	-4.10	0.003	S - H	21.10	<0.001
P - B	93.34	<0.001	W - H	-105.46	<0.001
S - B	17.00	<0.001	S - P	-76.35	<0.001
W - B	-109.56	<0.001	W - P	-202.90	<0.001
P - H	97.44	<0.001	W - S	-126.56	<0.001

Table S7 Pearson correlation r values between initial C:N ratio, % C, and % N content of residue types (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values are in parentheses. Correlations with p-values <0.05 are significant

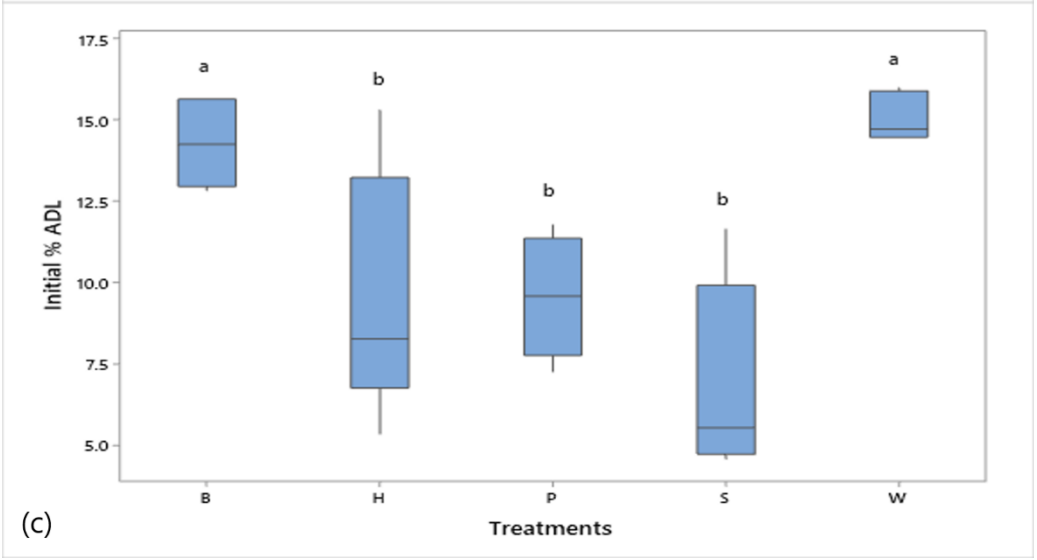
All types	N	C	***H* type**	N	C
C	-0.533			-0.814	
	(0,002)			(0.048)	
C:N ratio	-0.902	0.792		-0.993	0.879
	(<0.001)	(<0.001)		(<0.001)	(0.021)
***B* type**			***S* type**		
C	0.987			-0.296	
	(<0.001)			(0.569)	
C:N ratio	-0.982	-0.939		-0.977	0.495
	(<0.001)	(0.005)		(0.001)	(0.319)
***P* type**			***W* type**		
C	0.088			-0.146	
	(0.868)			(0.783)	
C:N ratio	-0.972	0.150		-0.988	0.296
	(0.001)	(0.777)		(<0.001)	(0.569)


Initial % NDF
60
55
50
45
40
35
30
bc
b
c
d
a
B
H
P
S
W
Treatments

(a)


Initial % ADF
45
40
35
30
25
20
b
c
d
d
a
B
H
P
S
W
Treatments

(b)


Initial % ADL
17.5
15.0
12.5
10.0
7.5
5.0
a
b
b
b
a
B
H
P
S
W
Treatments

(c)

Fig. S4 Initial (a) NDF, (b) ADF, and (c) ADL (%) content of the residues of the different treatments (*B, H, P, S,* and *W*) (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin). Means that do not share a letter are significantly different ($p<0.05$)

Table S8 Initial NDF, hemicellulose, ADF, cellulose, and lignin (%) content of the different residue types (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin). Means that do not share a letter are significantly different (p-value < 0.05)

	NDF	**hemicellulose**	**ADF**	**cellulose**	**lignin**
Types	**Mean ±StDev**	**Mean ±StDev**	**Mean ±StDev**	**Mean ±StDev**	**Mean ±StDev**
B	41.776 ±0.546^{bc}	15.575 ±0.413^{c}	26.201 ±0.412^{b}	11.902 ±1.382^{a}	14.299 ±1.348^{a}
H	43.503 ±0.254^{b}	19.588 ±0.156^{a}	23.915 ±0.165^{c}	14.26 ±3.83^{b}	9.66 ±3.76^{b}
P	40.475 ±1.163^{c}	20.608 ±0.890^{a}	19.868 ±0.312^{d}	10.296 ±1.853^{b}	9.572 ±1.857^{b}
S	34.623 ±1.223^{d}	14.617 ±0.543^{d}	20.006 ±0.745^{d}	13.03 ±2.73^{b}	6.97 ±2.98^{b}
W	59.367 ±0.983^{a}	17.036 ±0.537^{b}	42.331 ±0.618^{a}	27.236 ±0.663^{b}	15.095 ±0.743^{a}

Table S9 Tukey's post-hoc test for differences between types on initial % NDF, and % hemicellulose content of residues (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin). P-values <0.05 indicate significant differences

NDF			**Hemicellulose**		
Difference of Levels	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
H - B	2.99	0.050	*H - B*	11.60	< 0.001
S - B	-12.38	<0.001	*P - B*	14.13	< 0.001
W - B	30.44	<0.001	*S - B*	-3.22	0.031
P - H	-5.24	<0.001	*W - B*	4.53	0.002
S - H	-15.36	<0.001	*S - H*	-14.82	< 0.001
W - H	27.45	<0.001	*W - H*	-7.07	< 0.001
S - P	-10.13	<0.001	*S - P*	-17.35	< 0.001
W - P	32.69	<0.001	*W - P*	-9.60	< 0.001
W - S	42.81	<0.001	*W - S*	7.75	< 0.001

Table S10 Tukey's post-hoc test for differences between types on initial % ADF, and % cellulose content of residues (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. ADF = Acid Detergent Fiber (Cellulose and Lignin). P-values <0.05 indicate significant differences

ADF			**Cellulose**		
Difference of Levels	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
H - B	-7.28	<0.001	*W - B*	10.26	<0.001
P - B	-20.18	<0.001	*P - H*	-2.65	0.099
S - B	-19.74	<0.001	*W - H*	8.68	<0.001
W - B	51.39	<0.001	*W - P*	11.33	<0.001
P - H	-12.89	<0.001	*W - S*	9.50	<0.001
S - H	-12.46	<0.001			
W - H	58.67	<0.001			
W - P	71.56	<0.001			
W - S	71.13	<0.001			

Table S11 Tukey's post-hoc test for differences between types on initial % ADL (lignin) content of residues (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

Difference of Levels	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
H - B	-3.06	0.044	*S - H*	-1.77	0.418
P - B	-3.11	0.039	*W - H*	3.58	0.014
S - B	-4.82	0.001	*S - P*	-1.71	0.450
W - B	0.52	0.984	*W - P*	3.64	0.013
P - H	-0.06	1.000	*W - S*	5.35	<0.001

Table S12 Pearson correlation r values between initial NDF, ADF, hemicellulose, cellulose, lignin content of residues, and residue dry mass loss (%) considering all types together (N = 25). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Correlations with p-values <0.05 are significant

Sample 1	**Sample 2**	**Correlation**	**P-Value**
Hemicellulose	NDF	0.144	0.493
ADF	NDF	0.960	0.000
Cellulose	NDF	0.866	0.000
Lignin	NDF	0.636	0.001
Residue dry mass loss	NDF	-0.946	0.000
ADF	Hemicellulose	-0.139	0.509
Cellulose	Hemicellulose	-0.120	0.569
Lignin	Hemicellulose	-0.101	0.631
Residue dry mass loss	Hemicellulose	-0.068	0.747
Cellulose	ADF	0.900	0.000
Lignin	ADF	0.665	0.000
Residue dry mass loss	ADF	-0.928	0.000

Lignin	Cellulose	0.273	0.186
Residue dry mass loss	Cellulose	-0.834	0.000
Residue dry mass loss	Lignin	-0.619	0.001

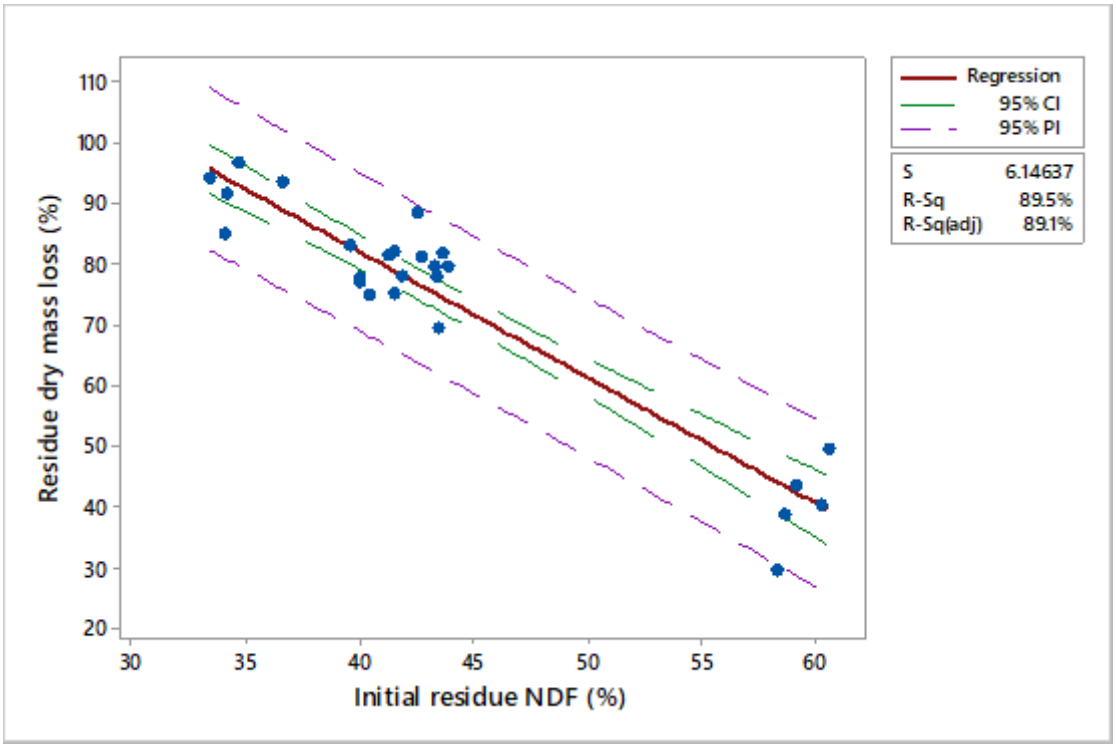


Fig. S5 Fitted line plot of linear regression analysis of residue dry mass loss (%) with initial residue NDF content (%) (red line). Confidence interval is enclosed by green dashed lines, and prediction interval by purple dashed lines. Confidence level = 95.0. Regression equation: residue dry mass loss = 165.01 - 2.073 NDF

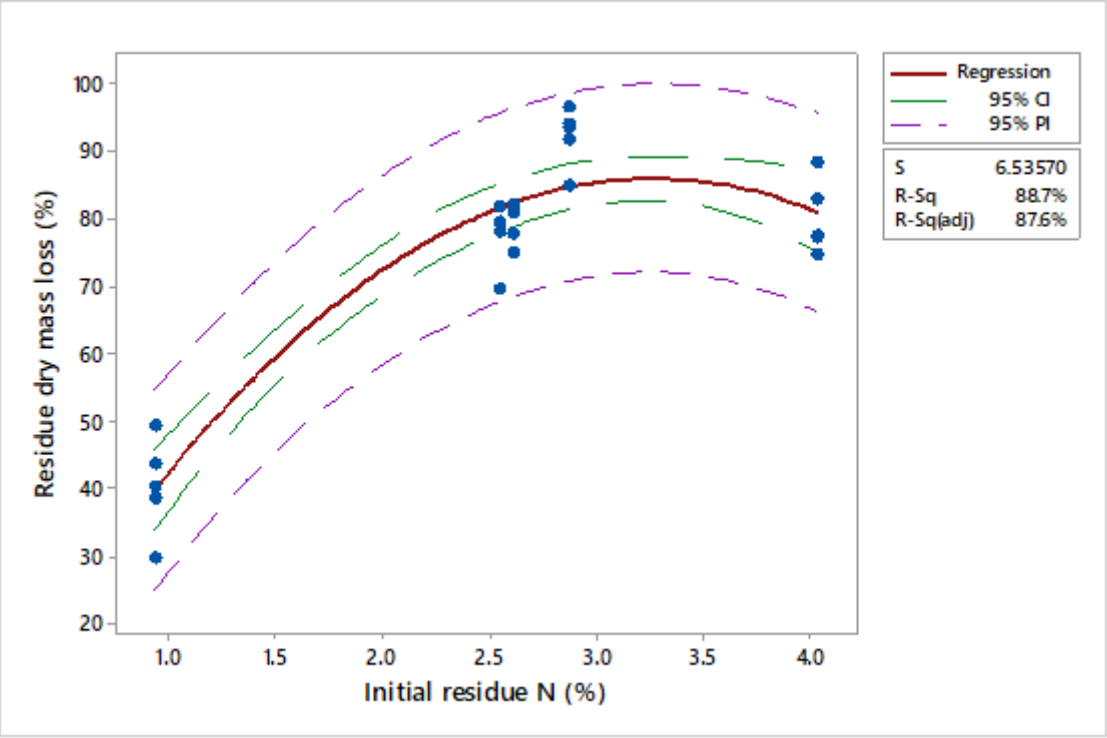


Fig. S6 Fitted line plot of polynomial (quadratic) regression analysis of residue dry mass loss (%) with initial residue N content (%) (red line). Confidence interval is enclosed by green dashed lines, and prediction interval by purple dashed lines. Confidence level = 95.0. Regression equation: residue dry mass loss = -5.016 +55.76 N – 8.534 N^2

Table S13 Soil solution pH of the different treatments (*B, Control (C), H, P, S,* and *W*) from 0-5 cm depth on day 69 and on day 195 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. The value of the bulk soil is also quoted for comparison. Means that do not share a common letter are significantly different (p<0.05)

Treatment	Mean ±StDev	Mean ±StDev
	Day 69	Day 195
B	7.4720 ±0.0918[a]	7.6540 ±0.0647[b]
C	7.5260 ±0.0702[a]	7.7940 ±0.1316[b]
H	7.4440 ±0.0680[a]	7.666 ±0.279[b]
P	7.3980 ±0.1383[a]	7.7420 ±0.1192[b]
S	7.4740 ±0.1680[a]	7.7180 ±0.1767[b]
W	7.4440 ±0.1508[a]	7.7420 ±0.0726[b]
Bulk	6.3600 ±0.0346[c]	

Table S14 Total % soil N at 0-5 cm depth for the different treatments (*B, Control (C), H, P, S,* and *W*) and for the bulk soil, on day 69 and on day 195 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means that do not share a letter are significantly different (p<0.05).

Treatments	Mean ±StDev	Mean ±StDev
	Day 69	Day 195
B	0.16620 ±0.01125[ab]	0.17273 ±0.01510[ab]
C	0.16052 ±0.00973[ab]	0.15199 ±0.01101[c]
H	0.16211 ±0.01214[ab]	0.16705 ±0.00465[bc]
P	0.17274 ±0.01972[ab]	0.17133 ±0.00302[ab]
S	0.15743 ±0.01197[b]	0.17378 ±0.00689[ab]
W	0.16554 ±0.01113[ab]	0.16493 ±0.00922[bc]
Bulk soil	0.18519 ±0.01071[a]	0.18519 ±0.01071[a]

Table S15 Tukey's post-hoc test, on total % soil N between treatments (*B, Control (C), H, P, S,* and *W*) or between treatments and bulk soil, from samples at 0-5 cm, on day 69 and on day 195 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

Difference of Levels	T-Value	Adjusted P-Value	Difference of Levels	T-Value	Adjusted P-Value
Day 69			**Day 195**		
S - Bulk	-3.44	0.027	*C - B*	-3.46	0.025
			C - Bulk	-5.54	<0.001
			W - Bulk	-3.38	0.031
			P - C	3.23	0.044
			S - C	3.64	0.017

Table S16 Tukey's post-hoc test, of soil NH_4^+ between treatments (*B, Control (C), H, P, S,* and *W*), at 50-55 cm depth, in interaction with time (day 69 and day 195 after mulch application) (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

Difference of Treatments*Time Levels	T-Value	Adjusted P-Value	Difference of Treatments*Time Levels	T-Value	Adjusted P-Value
(*C* d195) - (*B* d69)	-3.65	0.028	(*S* d69) - (*C* d195)	3.99	0.011
(*H* d195) - (*B* d69)	-4.24	0.005	(*W* d69) - (*C* d195)	3.80	0.018
(*C* d69) - (*B* d195)	3.46	0.047	(*H* d195) - (*H* d69)	-4.60	0.002
(*H* d69) - (*B* d195)	3.66	0.028	(*W* d195) - (*H* d69)	-3.47	0.045
(*S* d69) - (*B* d195)	3.63	0.030	(*P* d69) - (*H* d195)	3.91	0.013
(*W* d69) - (*B* d195)	3.44	0.049	(*S* d69) - (*H* d195)	4.57	0.002
(*C* d195) - (*C* d69)	-3.82	0.018	(*W* d69) - (*H* d195)	4.39	0.003
(*H* d195) - (*C* d69)	-4.40	0.003	(*W* d195) - (*S* d69)	-3.45	0.049
(*H* d69) - (*C* d195)	4.02	0.010			

Table S17 Soil content (mg/kg of oven dry soil) in elements (P, K, Mg, Fe, Mn, Zn, and Cu) between 0-5 and 20-25cm depths on day 69 after mulch application (N = 30). P-values <0.05 indicate significant differences. Fi = normality or equality of variances of data were not satisfied even after transformation so further investigation is needed.

Variable	Depth	Mean	StDev	F-value	p-value
P	0-5 cm	562.93	38.46	3.18	0.087
	20-25 cm	575.02	30.44		
K	0-5 cm	59.89	5.64	126.85	<0.001
	20-25 cm	83.86	12.67		
Mg	0-5 cm	53.88	9.63	277.72	<0.001
	20-25 cm	104.07	12.26		
Fe	0-5 cm	395.26	28.70	7.19	0.013

	20-25 cm	383.82	21.54		
Mn (fi)	0-5 cm	25.505	2.458	40.31	<0.001
	20-25 cm	22.492	2.351		
Zn (fi)	0-5 cm	22.921	1.811	6.79	0.016
	20-25 cm	24.240	2.818		
Cu	0-5 cm	4.056	0.796	23.95	<0.001
	20-25 cm	3.3242	0.2879		

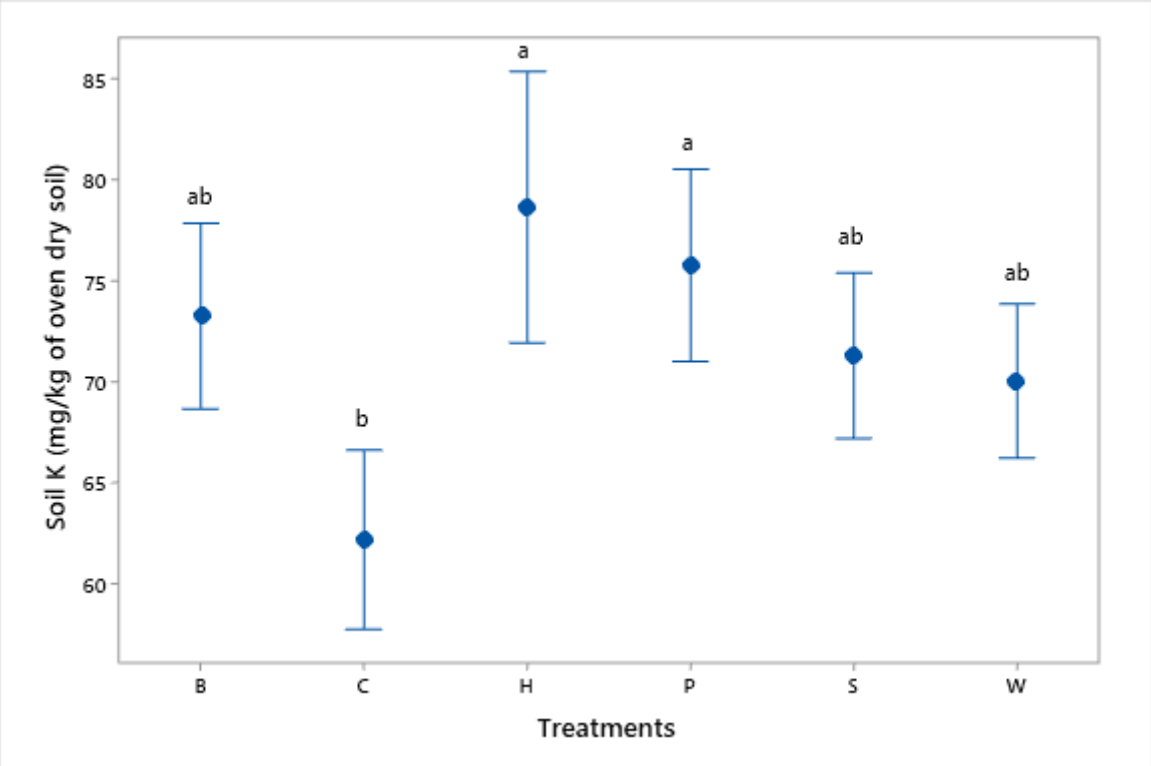


Fig. S7 Soil content (mg/kg of oven dry soil) in K for the different treatments (*B, Control (C), H, P, S,* and *W*), in combined samples from both 0-5 and 20-25 cm depths on day 69 after mulch application (N = 10). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means and bars of one standard error from the mean are depicted. Types that do not share a common letter are significantly different (p-value < 0.05)

Table S18 Comparisons, with Dunnett's test, of soil K between *Control (C)* and all other treatments (*B, H, P, S,* and *W*) on day 69 after mulch application at 0-5 and 20-25 cm depth (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

Depth	Treatments	Mean	StDev	Difference of Levels	T-Value	Adjusted P-Value
0-5 cm	*B*	61.41	3.58	*B - C*	3.20	0.016
	C	51.96	4.03	*H - C*	3.59	0.006
	H	62.57	2.56	*P - C*	3.56	0.007
	P	62.49	3.38	*S - C*	2.82	0.038
	S	60.30	5.99	*W - C*	2.92	0.031

	W	60.58	6.93			
20-25 cm	*C*	72.42	12.84	*H-C*	3.07	0.022
	H	94.72	19.05			

Table S19 Comparisons, with Dunnett test, of soil nutrients between the bulk soil and the treatments (*B, Control (C), H, P, S,* and *W*) on day 69 after mulch application at samples from both 0-5 and 20-25 cm depth. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

Elements	**Treatments**	**N**	**Mean**	**StDev**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
K	Bulk soil	4	225.36	6.89	*B* - Bulk	-17.20	<0.001
	B	10	73.26	14.53	*C* - Bulk	-18.45	<0.001
	C	10	62.19	14.03	*H* - Bulk	-16.59	<0.001
	H	10	78.65	21.24	*P* - Bulk	-16.91	<0.001
	P	10	75.78	15.06	*S* - Bulk	-17.42	<0.001
	S	10	71.30	12.95	*W* - Bulk	-17.56	<0.001
	W	10	70.05	12.08			
Mg	Bulk soil	4	124.94	8.44	*B* - Bulk	-2.79	0.027
	B	10	78.57	29.71	*C* - Bulk	-2.92	0.020
	C	10	76.5	32.9	*H* - Bulk	-2.73	0.032
	H	10	79.54	28.36	*P* - Bulk	-2.69	0.035
	P	10	80.24	26.16	*S* - Bulk	-2.86	0.023
	S	10	77.53	28.33	*W* - Bulk	-2.62	0.042
	W	10	81.43	26.58			
Fe	Bulk soil	4	357.2	21.9	*H* - Bulk	2.88	0.022
	H	10	401.44	30.74			
Cu	Bulk soil	4	2.9279	0.1351	*W* - Bulk	2.47	0.059
	W	10	3.941	1.085			

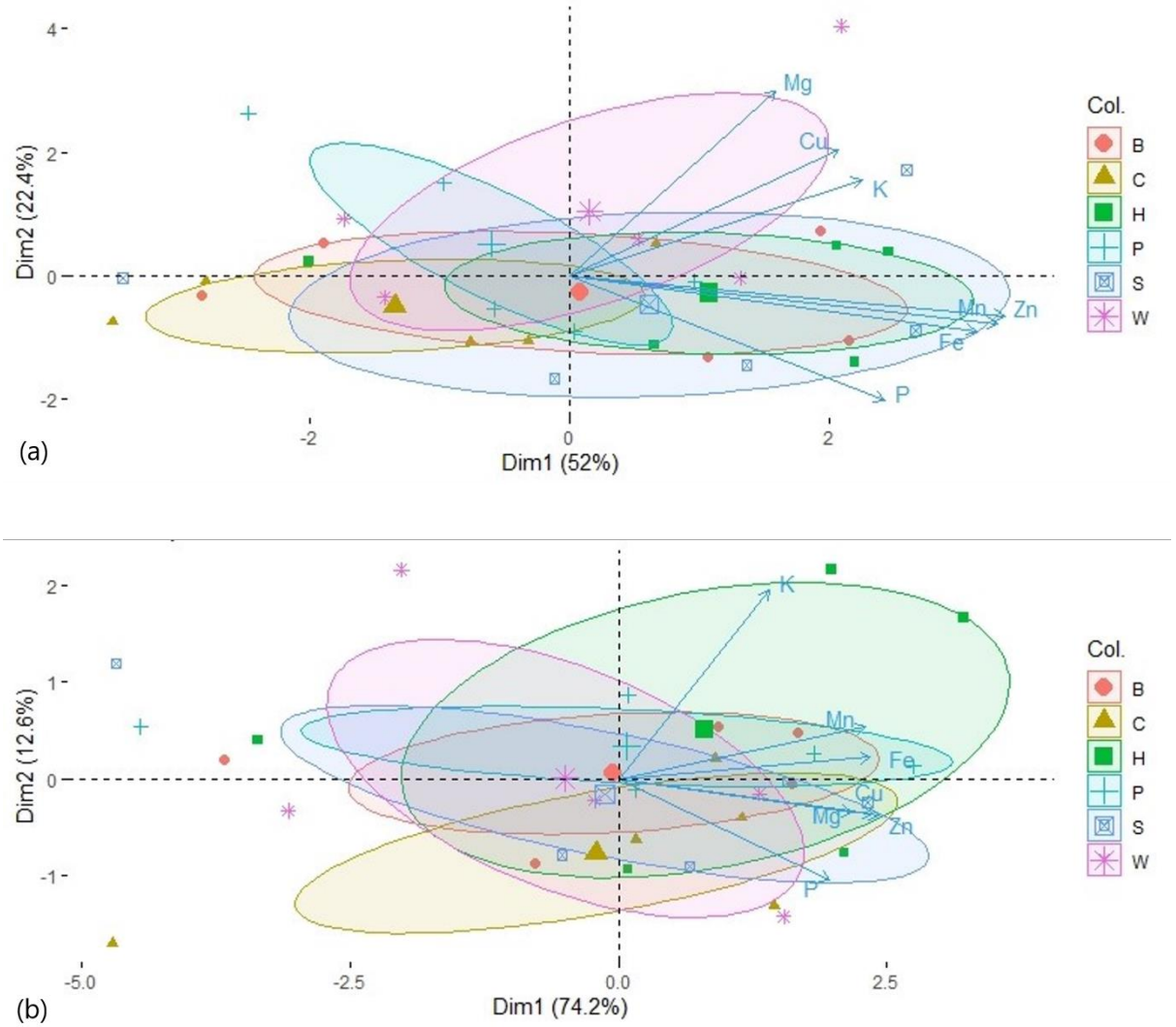


Fig. S8 PCA biplot of soil nutrients P, K, Mg, Fe, Mn, Zn, and Cu as variables for the treatments *B, Control (C), H, P, S,* and *W* concerning measurements (a) at 0-5 cm depth, and (b) at 20-25 cm depth, 69 days after mulch application. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Large points depict mean values, while ellipses depict confidence intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1 normalized score, and left axis represents Dim2 normalized score

Table S20 Phospholipid fatty acid (PLFA) compounds (nmol g-1 of freeze dry soil) present in soil microbial groups in the different treatments (*B, Control (C), H, P, S,* and *W*) at 0-5 cm depth, on day 69 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*.

Microbial groups	**Gram+ bacteria**		**Gram- bacteria**		**Fungi**		**Protozoa**		**Cyanobacteria**	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	904	880	100.5	56.5	482	437	54.9	41.4	14.24	7.79
C	591	367	68.35	20.47	351.7	204.0	23.25	8.28	6.586	2.140
H	654	540	74.2	27.4	389	289	37.08	19.17	11.26	5.00
P	979	564	109.0	44.5	544	290	42.51	20.81	15.72	10.59

S	2675	3456	244	273	1589	2184	108.8	135.7	36.3	47.9
W	696	407	75.07	18.18	384.3	170.0	22.77	4.65	6.898	1.215

Table S21 Phospholipid fatty acid (PLFA) compounds (nmol g-1 of freeze dry soil) present in soil microbial groups in the different treatments (*B, Control (C), H, P, S,* and *W*) at 0-5 cm depth, on day 195 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*.

Microbial groups	**Gram+ bacteria**		**Gram- bacteria**		**Fungi**		**Protozoa**		**Cyanobacteria**	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	598	408	108.4	62.5	532	303	28.91	14.47	10.97	4.81
C	791	621	131.1	94.6	556	333	31.78	17.91	11.45	3.51
H	946.4	177.1	168.7	39.4	822.3	165.5	45.73	9.23	16.22	4.79
P	669	410	122.5	43.3	616	337	34.45	19.24	10.64	5.06
S	1050	401	172.9	82.5	802	284	49.26	18.49	17.63	9.25
W	793	310	141.8	59.5	670	258	38.74	8.91	17.06	12.31

Table S22 Microbial biomass expressed in phospholipid fatty acid (PLFA) compounds (nmol g-1 of freeze dry soil) including all selected compounds considered for the estimation of the different microbial groups (Gram+ bacteria, Gram- bacteria, Fungi, Protozoa, and Cyanobacteria) for the different treatments (*B, Control (C), H, P, S,* and *W*) from soil samples at 0-5 cm depth, on day 69 and on day 195 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*.

	Day 69		**Day 195**		
Treatment	**Mean**	**StDev**	**Mean**	**StDev**	**% Difference**
B	1556	1413	1278	780	-17.87
C	1041	591	1521	1065	46.11
H	1166	872	1999	357	71.44
P	1690	912	1452	796	-14.08
S	4653	6094	2091	751	-55.06
W	1185	585	1661	589	40.17
All treatments	1882	2712	1667	748	-11.42

Table S23 Percentage difference (increase or decrease) of means of soil microbial biomass expressed in phospholipid fatty acid (PLFA) compounds (nmol g-1 of freeze dry soil) including all selected compounds considered for the estimation of the different microbial groups (Gram+ bacteria, Gram- bacteria, Fungi, Protozoa, and Cyanobacteria) for the different treatments (*B, Control (C), H, P, S,* and *W*) between day 69 and day 195 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*.

Treatments	**Gram+**	**Gram-**	**Fungi**	**Protozoa**	**Cyanobacteria**
B	-33.85	7.86	10.37	-47.34	-22.96
C	33.84	91.81	58.09	36.69	73.85
H	44.71	127.36	111.39	23.33	44.05
P	-31.66	12.39	13.24	-18.96	-32.32
S	-60.75	-29.14	-49.53	-54.72	-51.43
W	13.94	88.89	74.34	70.14	147.32

Table S24 Fungi: bacteria ratio of the different treatments (*B, Control (C), H, P, S,* and *W*), considering Gram+ and Gram- as bacterial biomass, on day 69 and on day 195 after mulch application. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*.

	Day 69	Day 195	
Treatments	fungi: bacteria	fungi: bacteria	Difference
B	0.48	0.75	0.27
C	0.53	0.6	0.07
H	0.53	0.74	0.21
P	0.5	0.78	0.28
S	0.54	0.66	0.12
W	0.5	0.72	0.22
All treatments	0.52	0.71	0.19

Table S25 Barley root dry mass (g) of the different treatments (*B, Control (C), H, P, S,* and *W*), and significant differences by Tukey's post-hoc test at samples from 0-10 cm depth at the end of the growing season (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

Treatments	**Mean**	**StDev**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
B	0.4650	0.0628	*W - H*	-3.23	0.037
C	0.4713	0.1527			
H	0.5448	0.1168			
P	0.4612	0.0992			
S	0.3621	0.0867			
W	0.3305	0.0884			

Table S26 Seed protein, N, and C content (%) of the different treatments (*B, Control (C), H, P, S,* and *W*) at harvest time (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Values that do not share a common letter are significantly different (p<0.05)

	Protein		N		C	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	12.051	0.649	2.1142	0.1138	44.568[a]	0.133
C	11.54	3.11	2.025	0.545	44.157[b]	0.232
H	10.953	0.477	1.9216	0.0836	44.192[b]	0.247
P	12.150	1.808	2.132	0.317	44.473[ab]	0.202
S	11.780	1.611	2.067	0.283	44.378[ab]	0.105
W	11.497	1.315	2.017	0.231	44.342[ab]	0.150

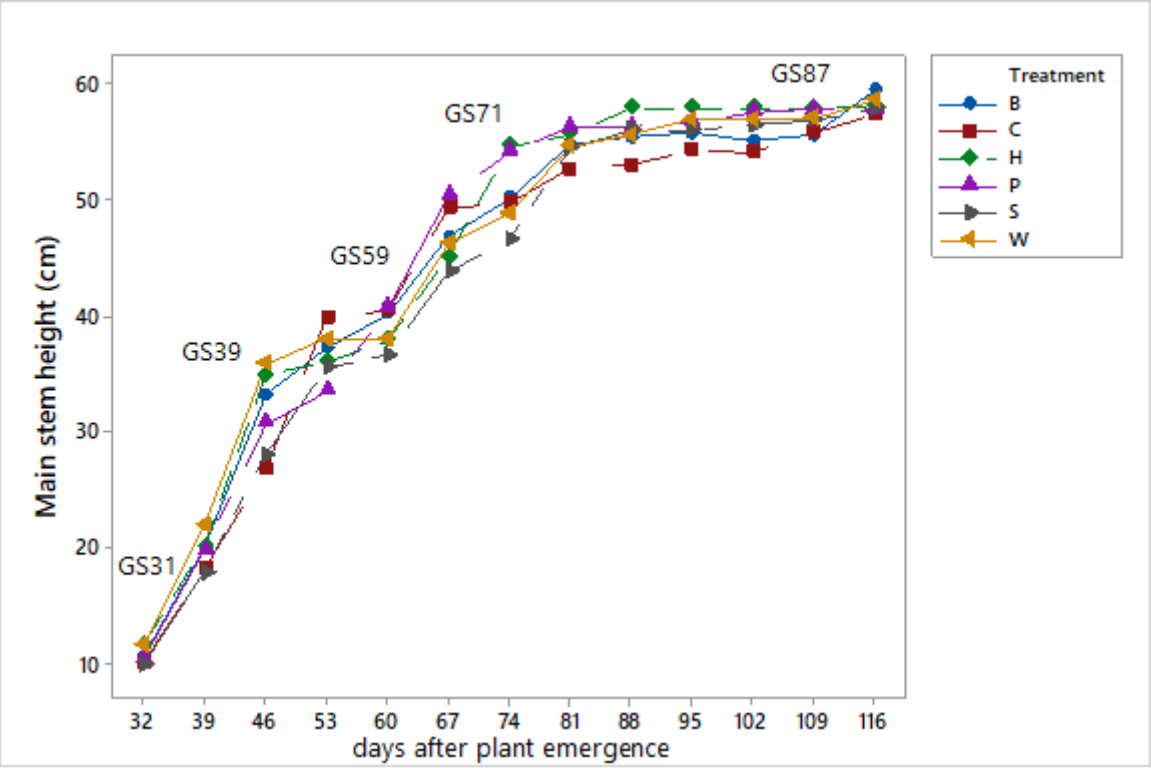


Fig. S9 Height (cm) of the highest stem of barley plants of the different treatments (*B, Control (C), H, P, S,* and *W*) measured from day 32 to day 116 after barley plant emergence. GS = Barley growing stage (Tottman et al., 1986). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*

Table S27 Classification of treatments (*B, Control (C), H, P, S,* and *W*) in descending order, according to their values in total % soil N at 0-5 cm depth as well as in soil NH_4^+ at 50-55 cm depth on day 69 and on day 195 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. The percentage increase or decrease from day 69 to day 195 is also quoted.

	Day 69	**Day 195**	**Differences % (day 69 to day 195)**					
Treatments			***B***	***C***	***H***	***P***	***S***	***W***
Total % soil N	*P>B>W>H>C>S*	$S^a>B^a>P^a>H^{ab}>W^{ab}>C^b$	3.93	-5.31	3.05	-0.82	10.39	-0.37
NH_4^+	*H>S>W>C>B>P*	*S>P>W>B>C>H*	-39.8	-43.5	-50	-33.8	-32.1	-39

Table S28 Soil macronutrients' (P, K, and Mg) content (mg kg-1 of oven dry soil) for the different treatments (*B, Control (C), H, P, S,* and *W*) from both 0-5 and 20-25 cm depths, on day 69 after mulch application (N = 10). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*

	P		**K**		**Mg**	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	571.21	31.06	73.26	14.53	78.57	29.71
C	558.82	27.89	62.19	14.03	76.5	32.9
H	590.21	27.52	78.65	21.24	79.54	28.36
P	561.0	40.9	75.78	15.06	80.24	26.16
S	583.8	34.3	71.30	12.95	77.53	28.33
W	548.9	35.5	70.05	12.08	81.43	26.58

Table S29 Soil micronutrients' (Fe, Mn, Zn, and Cu) content (mg kg-1 of oven dry soil) for the different treatments (*B, Control (C), H, P, S,* and *W*) from both 0-5 and 20-25 cm depths, on day 69 after mulch application (N = 10). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*

	Fe		**Mn**		**Zn**		**Cu**	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	388.81	25.20	24.04	3.37	23.692	2.408	3.552	0.465
C	381.71	23.69	23.571	2.356	23.203	2.548	3.728	0.657
H	401.44	30.74	24.87	3.50	24.027	2.371	3.788	0.812
P	387.06	25.27	23.430	2.302	23.191	2.661	3.4554	0.2906
S	390.6	32.4	23.88	3.31	24.233	2.832	3.678	0.685
W	387.62	17.30	24.206	2.356	23.137	2.188	3.941	1.085

Chapter 4

Paper 3

High quality plant-based mulch may increase soil fertility early, but mulch of lower quality contributes more at later stages of decomposition in field conditions.

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Authors' contributions: Conceptualization, D.G.; Experimental design, D.G., and M.L.; Experimental work, D.G.; Laboratory analyses, D.G.; Data analysis, D.G.; Data curation, D.G., M.L., and M.T.; Supervision, M.L., and M.T.; Writing – original draft, D.G.; Writing – review and editing, D.G., M.L., and M.T.

Abstract

Plant-based mulch in the field provides physical protection of the soil throughout the cultivation period but also may affect soil chemistry and biology. However, little attention has been given in the effect of mulch diversity on soil chemistry and biology at later stages of decomposition, to support crop growth. We investigated the effect of diversity and quality of whole-plant residue mulches of mixes on N mineralization, and on soil microbial community 25, 52, or 90 days after mulch application, and on final quality of residues mixed with crop residues at the end of the growing season in field conditions. Treatments included mixtures of 17, 12, 6, 1 plant species, wood chips, and a control treatment without residues, in a fully factorial field experiment. The C:N ratio of final residues was reduced, provided mulch of higher quality in mixtures with crop residues in comparison to crop residues alone. N mineralization was significantly higher in residues of higher quality. The effect of residue quality on soil microbial biomass on day 52 was not significant. Residue C and lignin content inversely affected ear length, and increased seed weight. Generally, whole-plant residues as mulch in field conditions can influence N mineralization, crop quality, and possibly soil microbial community at later stages of decomposition. The remaining residues combined with crop residues can improve their quality. Further research is needed with continuous applications of whole-plant residues in field conditions to determine optimal amounts, and sufficient number of applications to maximize benefits.

Key words: whole-plant mulch, residue mixtures, residue quality, N mineralization, soil microbes, quality of crop production, late stages of decomposition.

Introduction

Plant-based mulches have beneficial effects on soil physical properties (Prats *et al.*, 2017; (Su et al., 2014); Siczek and Lipiec, 2011; Blanco-Canqui *et al.,* 2011; Jordán *et al.,* 2010), although negative effects under conditions have been reported as well (Halde *et al.,* 2015). They also influence soil chemistry and biology, but these effects have not been thoroughly studied. Most studies were focused on C, N, and P mineralization (Oelmann *et al.*, 2017; Ch'Ng, Ahmed and Majid, 2014; Singh and Khind, 1992). The effect of mulching in the concentration of soil carbon and nitrogen is not readily obvious (Calderón, Nielsen, Acosta-Martínez, Vigil, & Lyon, 2016). It can take up to nine or ten years to notice a considerable increase in soil C (Olson *et al.,* 2010; Peterson *et al.*, 1998). Release of C is increasing by increasing the amount of residues, but N may be initially immobilized by soil microbes, especially in a N-deficient soil (Dietrich et al., 2017). N immobilization usually coincides with crop early vegetative stages leading to N deficiency in plants (Siczek & Lipiec, 2011), the most common deficiency in plant production. N mineralization is a favourable index to estimate the rate of decomposition of a particular substrate, as N is mineralized in large quantities relatively to other essential nutrients. The quality of the substrate plays important role in the fate of N cycling. Plant-based residues of high quality, usually with C:N ratio less than 24, are considered to lead to direct N mineralization (Hadas *et al.,* 2004). Legume plants due to their fixation capacity have low C:N ratio and are included in most cover crop management systems to enrich soil N (Wendling et al., 2017). Residues of lower quality (high C:N ratio) can lead to initial N immobilization, especially in N-poor soil conditions (Siczek and Lipiec, 2011; Frouz *et al.*, 2015). However, it has been suggested that C:N ratio could be used as an indicator for residue decomposition only in N-poor soils (Bonanomi et al., 2017), or independently of soil conditions when C:N ratio is very high (> 35) (Brust, 2019). C and N mineralization have been found to be influenced not only by C:N ratio but also by other endogenous factors of residues like N/P ratio, and recalcitrant substances like cellulose, lignin, and polyphenols (Zhonglu *et al.*, 2015; Vahdat *et al.,* 2011; Austin and Ballaré, 2010). Hemicellulose and cellulose are intermediately decomposable fractions, and lignin is a slowly decomposable fraction, therefore, all these three constituents should be considered when measuring residue recalcitrance (Hadas *et al.*, 2004). However, little attention has been given in C and N mineralization at later stages of decomposition. Slow release of nutrients for longer period may be equally or even more beneficial than a faster early release, depending on the needs of cash crop. Supply of large amounts of N in cereal growing stage of rapid stem elongation, GS32 to GS39 (Tottman *et al.,* 1986), is critical to avoid yield loss (Boyle, 2017).

Soil microbial composition and activity are also influenced by residue quality. Bacteria are favoured by high quality residues than fungi due to higher N content in their body (Rousk & Bååth, 2007). Total microbial biomass, and gram negative bacteria biomass was higher after addition of low C:N ratio residues, while the opposite was true for fungi and gram negative bacteria (Liang, Yuan, Yang, & Meng, 2017). Fungi are more competitive in residues of low quality and high recalcitrance (Rousk & Bååth,

2011). Fungi are more capable in exploitation of residue mulch because they develop hyphae to reach residues over the soil surface and to translocate nutrients (Ambus & Jensen, 1997). Contrary bacteria are favoured by the contact of residues with the soil (Barreiro *et al.,* 2016). As decomposition proceeds, residue recalcitrance increases, increasing fungal: bacterial ratio at the end of the growing season. In a three year experiment of no-till with plant residues of low quality, fungi dominated initially and bacteria thereafter, while with high quality residues bacteria dominated from the beginning (Frasier et al., 2016). Bacteria are more sensitive to changes in soil solution pH and require higher values than fungi. However, there were cases that an alter in fungi: bacteria ratio was not in line with expectations considering different environmental factors (Strickland & Rousk, 2010). Fungi are favoured in a low soil solution pH irrespective of substrate quality (Grosso et al., 2016). Residue mulch results in decrease of pH, raising the fungal: bacterial ratio (Singh & S. Khind, 1992). Soil organisms other than fungi and bacteria like nematodes, protozoa, and earthworms also participate in N immobilization and mineralization. Their contribution is significant in residue C:N ratio over 30, and optimal among 20 and 30 (Frouz et al., 2015).

There were cases where residue mulch has been found to affect seed quality and yield. Mulch resulted in higher seed protein content of soybean (Siczek & Lipiec, 2011) or of maize grain (Awopegba et al., 2017). A sufficient amount of residues can increase yield by weed suppression (Ranaivoson, Naudin, Ripoche, Rabeharisoa, & Corbeels, 2018). Opposite, a low amount of mulch can allow weed growth and subsequently reduce yield (Halde *et al.,* 2015).

Most research on the effect of plant-based mulch on soil chemical and biological properties concern incubation experiments with single plant species, finely grounded or chopped, focused on the early stage of decomposition. There is a gap in research, in field conditions, on diverse mixtures of whole-plant residue mulch (one cut near soil surface), and their effect on soil chemical and biological properties at later stages of decomposition. Residues in field conditions are distributed heterogeneously and their chemical and biological behaviour are subject to spatial variation. A decrease in microbial activity and N mineralization is expected by heterogeneous distribution of residues in field due to limited diffusion of available N from bulk soil to residue sphere, soil in immediate contact that is directly affected by residues (Magid *et al.,* 2006). Whole-plant residues are decomposed slower than chopped residues (Singh & S. Khind, 1992), providing effective physical protection on soil surface for longer period. Nutrient release has been found to be higher in smaller particle size residues, but without deleterious effect on crop yield (Reichert et al., 2015). Therefore, whole-plant residues are likely to maintain a relatively higher capacity of nutrient release at the end of cultivation period, so as in combination with cash crop residues they can constitute a satisfactory substrate for adequate nutrition for the successive cash crop (legacy effect). Legacy effect is attributed in exchange of nutrients between microbes decomposing the initial and the fresh organic matter (Zheng & Marschner, 2017). Residue mulch of mixtures of plant species can improve soil microbial

diversity due to enzymatic specificity of soil microbes in different substrates (Fontaine *et al.,* 2003). Appropriate handling of mixtures produced by plants previously cultivated in the field as cover crops can provide both N supply and N retention services, avoiding $N)_3^-$ leaching (White et al., 2017).

We hypothesized that, in field conditions, (i) the remaining residues in combination with crop residues at harvest, produce mulch of higher quality than that of the crop residues alone, (ii) residues of higher initial quality (low C:N ratio, high N, low recalcitrant substances) result in higher N mineralization than residues of lower quality at the later stages of decomposition, (iii) soil microbial biomass, at a later stage of decomposition, and crop quality (including crop morphology) are affected by residue diversity or quality.

Methodology

Experimental design and materials

The site of the experiment was at the Crop Research Unit of the University of Reading in Sonning, Reading in UK, GPS coordinates: 51°28'34.223'' N, 0°54'10.156'' W (OSMF, 2019). It was a field experiment with plot of 1.6 m^2 (2 m x 0.8 m) as experimental unit in 5 replications. There were 30 plots in total and the soil was a sandy loam (60% sand, 34% loam, 6% clay – USDA classification). The experiment started in March and completed in September 2019. The mean monthly temperatures during this period were 9, 11, 13, 16, 19, 19, and 15°C, respectively (WWO, 2019). The plots received only rainfall water and the mean monthly rainfall ranged between 41.6 mm in April and 148.5 mm in June (WWO, 2019).

All plots were sown with spring wheat (*Triticum aestivum, var. Paragon*) on 22 March 2019 and harvested after 157 days. Plant residues of five different mixtures (Types) of plant species were applied as mulch at each plot (experimental unit). A *Control* treatment *(C)* without residues was also included. The residue Types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass™ ***(S)*** (6 species), Biomix™ ***(B)*** (12 species), Herbal™ ***(H)*** (17 species), and Wood chips (***W***) (Table S1). Therefore, the 6 treatments were: ***H, B, S, P, W, and C***. These residue types were suitable to address our hypothesis because they included residue mixtures with different characteristics. Residue characteristics include both residue diversity and functional traits. Residue diversity may concern either the species composition or the species richness which is the number of species participating in a residue mixture. The functional traits of residues may concern either the chemical composition, which determines the residue quality, or the morphological features of residues (Santonja *et al.*, 2018; Hattenschwiler et al., 2011). All residues, except *W*, were collected from field plots on 20th May 2019 as whole plants cut once on the level of soil surface and stored at 4^0C until application. The encoding $P_{(1)}$, $S_{(6)}$, $B_{(12)}$, and $H_{(17)}$ has been adopted where it was considered necessary to indicate the number of species participating in the different residue types collected from field plots. Wood chips consisted of shredded tree residues (less than 10 cm) of branches and twigs with their leaves, collected from Harris Garden from the University of

Reading campus on 24th May 2019 and stored at 4°C until application. All types of residues were applied as fresh mulch in the field plots in quantities corresponding to 2.3 kg of dry mass per plot on 31st May 2019 (Fig. S1, S2, and S3). Not any kind of fertilization, artificial or organic, other than the mulch was applied on plots during experiment.

Sampling protocol

Soil samples were taken from the field plots at four time-points. The first was 15 days prior to mulch application on 16th May 2019, from 0-5 cm depth, to estimate soil solution pH, and % total soil C and N of the bulk soil (initial soil, prior to its use in rhizotrons). The second was 25 days after mulch application, when the crop plants were at the growing stage of GS60 to GS65 (beginning to half way of anthesis) (Tottman et al., 1986), from the depth of 30-45 cm to estimate soil content in NH_4^+ and NO_3^-. Under the term NO_3^- both soil NO_2^- and NO_3^- contents were included. The third was 52 days after mulch application, when crop plants were at the growing stage of GS75 to GS80, from 0-5 cm depth, for PLFA analysis or to estimate soil solution pH, and % total soil C and N. The fourth was 90 days after mulch application, at the end of the growing season (harvest time), from the depth of 30-45 cm to estimate soil content in NH_4^+ and NO_3^-. All soil samples were air-dried, sieved to <2 mm and stored at room temperature until analysis, except those for PLFA analysis which were sieved to <2 mm, freeze dried by a Super Modulyo freeze dryer (Edwards company) and stored at -20°C until analysis.

Seeds and other parts of wheat plants from all plots were collected from 26th to 28th August 2019, 87 to 89 days after mulch application. Plant residues were collected 118 days after mulch application, in a mixture together with residues of harvested wheat plants. All plant materials were dried at 80°C for 48 to 72 hours to constant weight.

Soil and plant material analysis

Measurements were conducted on plant residues, on wheat plants, and on soil samples to estimate physical and chemical properties.

Total C, N and protein of seeds, plant residues, and soil samples were estimated with combustion by LECO CHN 628 analyser (LECO Corporation). Dry mass of plant residues and seeds was estimated by drying at 80°C for 48 to 72 hours to constant weight and of soil samples at 105°C for 24 hours. ANKON 2000 Fibre Analyser (ANKON Technology) was used to measure recalcitrant substances of plant residues according to ANKOM Technology protocols (ANKON Technology, 2020). Samples were first dried at 80°C to constant weight and milled to < 1mm in a Fritsch grinder by Glen Creston Ltd. Plant residue fiber analysis was consisted of 3 successive processes to determine the % Neutral Detergent Fiber (NDF) (Hemicellulose, Cellulose and Lignin), the % Acid Detergent Fiber (ADF) (Cellulose and Lignin), and the % Acid Detergent Lignin (ADL) (Lignin). The % Cellulose was estimated by subtracted the % ADL from the % ADF, and the % Hemicellulose by subtracting the % ADF from the % NDF. Neutral

Detergent Solution (NDS), Alpha-amylase and anhydrous sodium sulfite were used as reagents for % NDF, Acid Detergent Solution for % ADF, and Sulfuric acid 72% by weight for % ADL.

Soil solution pH was measured with a calibrated pH meter. Air dry soil samples of 10 g, sieved to < 2 mm, were put in centrifuge tubes where 25 mL of ultra-pure water were added, and the tubes were shaken for 15 minutes on an end over end shaker before measurement (Blakemore, Searle, & Daly, 1987). Available N in soil samples was estimated by KCl extraction, according to standard protocol (Great Britain. M.A.F.F., 1986), colorimetrically, in a San Continuous Flow Injection Analyzer (SKALAR Instruments) using 40 g of air-dry soil in 200 ml 1M potassium chloride solution.

Fungi: Bacteria ratio in freeze dry soil samples of 3 ±0.1 g was estimated with Phospholipid Fatty Acid (PLFA) analysis as described by Sizmur et. al (2011). Samples were analysed by an Agilent 6890N Network GC System Gas Chromatographer (Agilent Technologies). Methyl tetradecanoate (C14:0) internal standard, Methyl nonadecanoate C19:0 Internal standard, Bacterial Acid Methyl Ester (BAME) Mix Quantitative standard, and Supelco 37 Component (FAME) Mix Quantitative standard were used. The chromatograms were elaborated by Agilent ChemStation – G2190BA – B04.03 software (Agilent ChemStation company). The Retention Time Window was set to 0.10 min and 1.5%, with Methyl tetradecanoate (C14:0) and Methyl nonadecanoate (C19:0) internal standards as Reference compounds. Soil samples were freeze dry in an Edwards Super Modulyo freeze dryer (Edwards company) soon after their collection and stored to -20^{0}C until the analysis.

Two air dried and sieved to <2mm soil samples were used to estimate soil texture with a hydrometer by adding 50 ml sodium hexametaphosphate solution (SHMP) at 50 g/l to 40 g soil subsamples (Bouyoucos, 1962).

Statistical analysis:

All statistical analyses were carried out in Minitab 19 (Minitab, LLC) except Principal Component Analysis which was conducted in R-Studio (RStudio, PBC). All measurements from a treatment were averaged to obtain a single mean value, the unit of replication of this study being the plot (n=5), and α = 0.05 was used to denote significance. One-way ANOVA and General Linear Model were used to detect differences between treatments in the case of one and two factors, respectively. Measurements repeated in time (day 25 and day 90 after mulch application) were analyzed by Mixed Effects Model with plots as random factor and residue type, and time as fixed factors. All data subjected to analyses of variance were tested for normality and homogeneity using Darling-Anderson and Levene tests. When these conditions were not satisfied, Kruskal-Wallis test was conducted in the case of one-way ANOVA, while in the other cases transformation of data with $\log_{10}$ or Box-Cox transformation with optimal or rounded λ was attempted. Where there were significant differences a Tukey post-hoc test was conducted. Comparisons with control treatment were made using the Dunnett test. Data was separated and analyzed at a specific sampling time, where normality or equality of variances were not

satisfied even after data transformation. Descriptive statistics included means and standard deviations rather than standard errors unless it was otherwise stated.

Pearson and Spearman correlations were used to assess the significant correlations between variables related with initial residue chemical composition (C, N, recalcitrant substances, and C:N ratio), and with seeds and ears (seed C, seed C:N ratio, ear length, dry mass of 1000 seeds, and dry mass of ears with seeds). Furthermore, regression analysis was conducted to examine the significance and the degree of the effect of a single independent variable to a response variable of particular interest in cases where there was a significant correlation (wheat ear length vs initial residue C, dry mass of 1000 seeds vs initial residue C). Homogeneity of variance of the data was tested with Levene test, and normality of data distribution with Anderson-Darling test. The Spearman correlation was used when normality or equality of variances of data in regression analysis were not satisfied.

Multivariate analysis was also conducted using Principal Component Analyses (PCAs) to assess differences between treatments in several variables simultaneously, and the relations of the treatments with those variables concerning measurements on soil microbial biomass or on barley plants.

Results

Effect on residue quality

The initial C:N ratio, C, and N of plant residues amended to the experimental plots was significantly different in all combinations between residues of different diversity (types), except of initial C between $S_{(6)}$ and $H_{(17)}$ types (N = 6, F = 6560.44, p-value < 0.001 for initial C:N ratio), (N = 6, Kruskal-Wallis test, H-value = 27.10, p-value < 0.001 for C), and (N = 6, F = 13333.62, p-value < 0.001 for N), (Fig. 1, Tables 1, S2, S3). The correlation matrix between initial residue C:N ratio, C, and N content showed significant strong negative correlations between N and C:N ratio for each of the residue types (p-value <0.05) but no significant correlations between C and N (p-value >0.05) (Table S4).

Both the hemicellulose (N = 5, F = 308.95, p-value < 0.001) and the cellulose (N = 5, Kruskal-Wallis test, H-value =17.69, p-value = 0.001) content was significantly different between diverse residues (Tables S5, and S6). There were significant differences between diverse residues in initial NDF (N = 5, F = 591.93, p-value < 0.001), in initial ADF (N = 5, F = 2299.77, p-value < 0.001), and in initial ADL content (N = 5, Kruskal-Wallis test, H-value = 12.15, p-value = 0.016) (Fig. 2). In all cases *W* type had the highest value, significantly different from all the others. There were not significant differences in ADL (lignin) content between $H_{(17)}$, $B_{(12)}$, $S_{(6)}$, and $P_{(1)}$ types (Tables 1, S5, and S6). Significance of differences was similar between diverse residues in both NDF (hemicellulose, cellulose, and lignin), and ADF (cellulose and lignin) content (Table 1). There was significant strong positive correlation (r = 0.927, p-value < 0.001) between NDF and ADF, and significant positive between ADF and lignin content (r = 0.550, p-

value = 0.004). Moreover, hemicellulose was significantly negatively correlated with both ADF (r = -0.507, p-value = 0.010) and lignin (r = -0.788, p-value < 0.001) (Table S7).

Table 1 Classification of residue types or treatments (*B, Control (C), H, P, S,* and *W*) in descending order of their initial or final (118 days after mulch application) mean values according to quality characteristics. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Types or treatments of the same property that do not share a common letter are significantly different (p-value < 0.05)

Properties	**Residue types**	**Properties**	**Treatments**
Initial C:N ratio	$W^a>H^b>B^c>S^d>P^e$	Final C:N ratio	$W^a>C^{ab}>H^{bc}>B^{bc}>S^{bc}>P^c$
Initial % C	$W^a>S^b>H^b>B^c>P^d$	Final % C	$W<C<S<H<B<P$
Initial % N	$P^a>S^b>B^c>H^d>W^e$	Final % N	$P>S>B>H>C>W$
Initial % NDF	$W^a>H^b>B^b>S^c>P^c$	Final % NDF	$H^a>S^a>W^{ab}>B^{ab}>C^{ab}>P^b$
Initial % ADF	$W^a>H^b>B^b>S^c>P^c$	Final % ADF	$W>S>H>B>C>P$
Initial % ADL	$W^a>P^b>S^b>B^b>H^b$	Final % ADL	$B>C>H>P>S>W$
Species richness	$H>B>S>W>P$		

The final quality of residues was determined by samples received at the end of the growing season, 118 days after mulch application. Wheat plants had already been killed, so residue samples were consisted of both residues from treatments and from wheat plants. The final C:N ratio was significantly different between treatments (N = 5, F = 8.18, p-value < 0.001). *W* treatment had significantly higher value (36.03 ±5.33) than all other treatments except *Control* (32.38 ±2.97) which was consisted only of residues from wheat plants. $P_{(1)}$ treatment had the lowest value (19.553 ±0.621) (Fig. 1, Tables 1, S8, and S9). Contrary, both the final C (N = 5, F = 1.47, p-value = 0.237) and the final N (N = 5, F = 2.50, p-value = 0.058) content of residues were not significantly different between treatments (Fig. 1, Tables 1, and S8). The correlation matrix between final residue C:N ratio, C, and N content showed significant strong positive correlation between N and C only for $P_{(1)}$ treatment (r = 0.991, p-value = 0.001), and significant negative correlations between N and C:N ratio in $S_{(6)}$, $B_{(12)}$, and $H_{(17)}$ treatments (Table S10).

The final cellulose content was significantly different between treatments (N = 5, F = 3.27, p-value = 0.022), but the final hemicellulose was not significantly different (N = 5, T-value = -2.81, adjusted p-value = 0.090). Particularly, cellulose content in $P_{(1)}$ treatment was significantly lower than in $H_{(17)}$, *W*, and *Control* treatments (Table S11). The final NDF content of residues was significantly different between treatments (N =5, F = 2.68, p-value = 0.046). $P_{(1)}$ (58.65 ±2.60) treatment was significantly lower than $H_{(17)}$ (65.81 ±2.63, N = 5, T-value = -3.13, p-value = 0.046) and $S_{(6)}$ (65.74 ±3.97, N = 5, T-value = 3.10, p-value = 0.049) treatments (Tables 1, S12). There were no significant differences between treatments in final ADF (N = 5, F = 1.30, p-value = 0.295), and in final ADL content (N = 5, F =

0.46, p-value = 0.805) (Fig. 2, Tables 1, S12). There were significant positive correlations between final NDF and ADF (r = 0.868, p-value <0.001), and between ADF and ADL content (r = 0.699, p-value <0.001), Also, there were significant negative correlations between hemicellulose and ADL (r = -0.676, p-value <0.001) and between cellulose and ADL (r = -0.651, p-value <0.001), and significant positive correlation between hemicellulose and cellulose (r = 0.790, p-value <0.001) (Table S13).

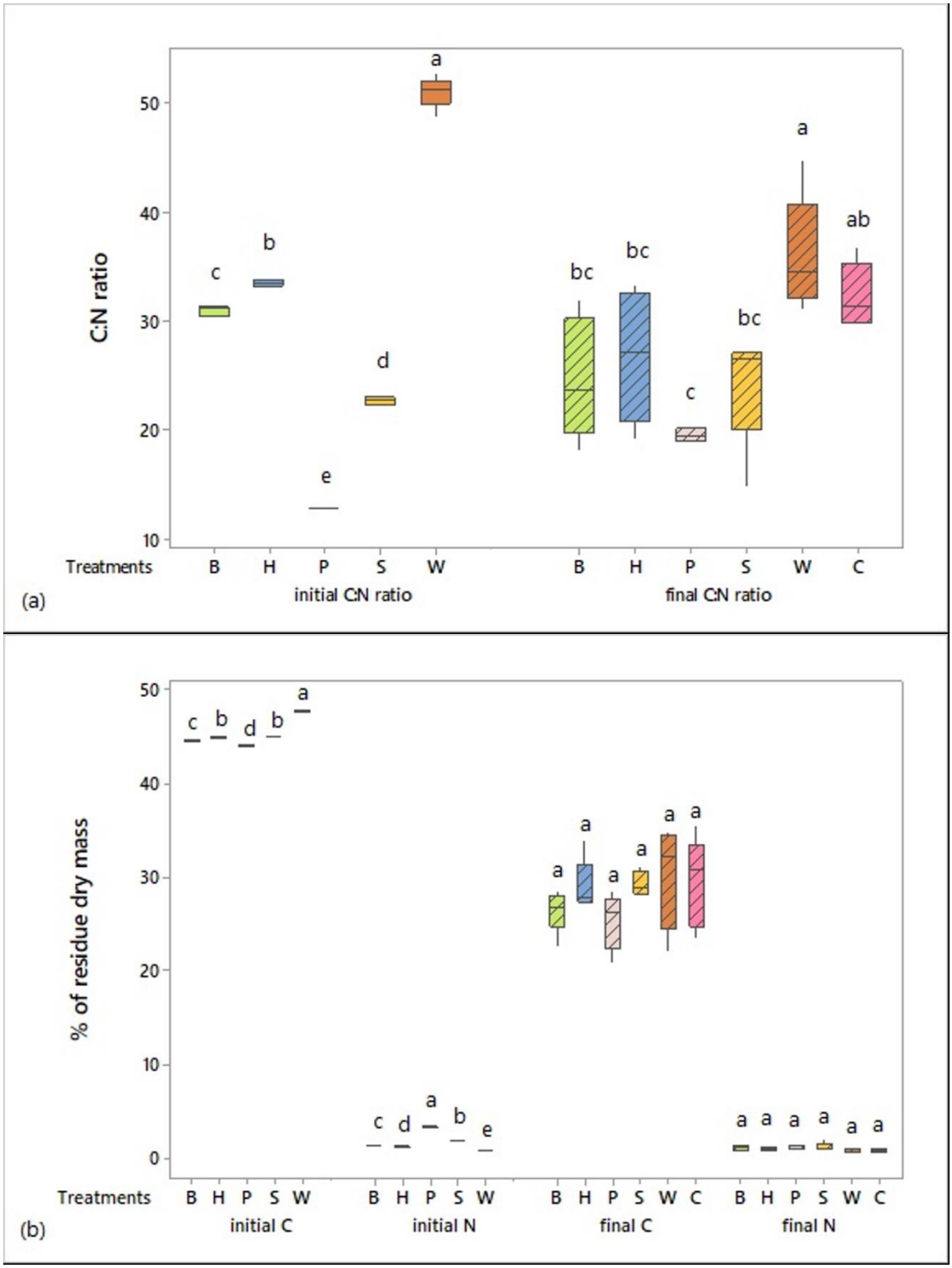

Fig. 1 Box and Whiskers plots of (a) initial and final % C, (b) initial and final % N, and (c) initial and final C:N ratio of residues of the different treatments (*B, Control (C), H, P, S, and W*) (N = 6). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means of the same variable that do not share a common letter are significantly different ($p<0.05$)

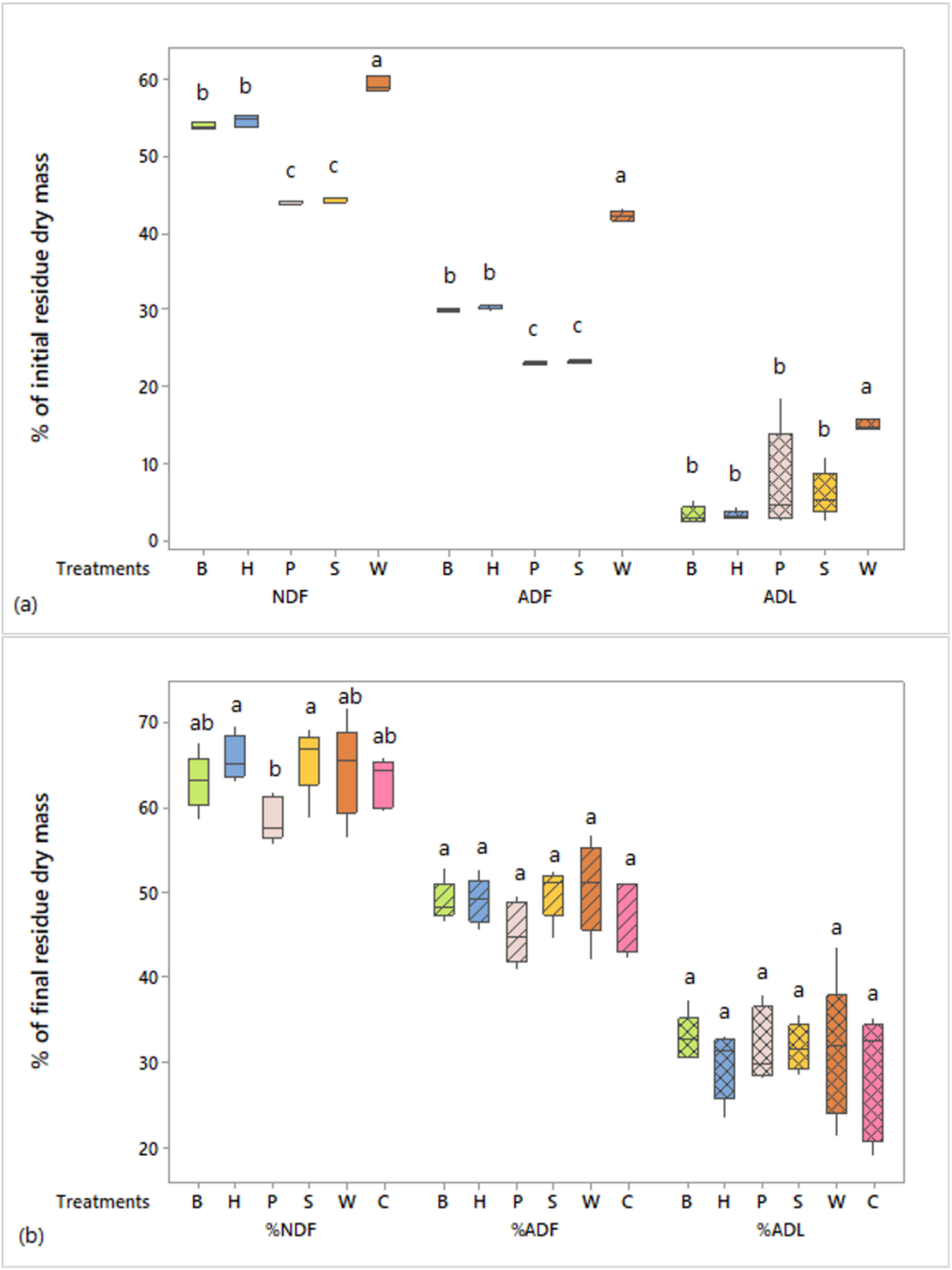

Fig. 2 Box and whiskers plots (a) of **i**nitial, and (b) of final % residue NDF, ADF, and ADL content of the different treatments (*B, Control (C), H, P, S, and W*) (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin). Means of the same variable that do not share a common letter are significantly different ($p<0.05$)

Effect on soil chemistry

Soil solution pH at 0-5 cm depth, 52 days after mulch application, was not significantly different between treatments (N = 5, F = 0.90, p-value = 0.511, Table 2). However, a reduction in pH was noticeable in all treatments in comparison with bulk soil (initial soil prior to the start of the experiment). *Control* had the lowest reduction (1.1%), and $P_{(1)}$ treatment the highest (8.2%) (Table S14). The same samples have been used to estimate total soil C, and N. There were no significant differences between treatments in total soil N (N = 5, F = 0.35, p-value = 0.879), and in total soil C content (N = 4, F = 0.78, p-value = 0.574). However, it is clear all treatments resulted in increased values in comparison with the bulk soil, although not significantly different (N = 5, F = 0.59, p-value = 0.734 for total soil N, and F = 0.57, p-value = 0.754 for total soil C). *Control* had of the highest values in both elements (Table S14).

Soil NH_4^+ and NO_3^- in mg kg^{-1} of oven dry soil were evaluated from samples at 30-45 cm depth both 25 days and 90 days after mulch application. Soil NH_4^+ was not significantly different between treatments, considering all samples from both time-points (N = 10, F = 0.49, p-value = 0.777). However, values from day 25 (4.184 ±0.638) were significantly different than those from day 90 (2.4356 ±0.3733) (n = 30, F = 238.17, p-value < 0.001). Further analysis considering soil NH_4^+ content at each time-point separately confirmed there were not significantly differences between treatments on both day 25 (N = 5, F = 0.50, p-value = 0.776) and day 90 (N = 5, $\log_{10}$ transformation of data, F = 0.61, p-value = 0.695). *Control* and $P_{(1)}$ treatment which had the highest values of soil NH_4^+ on day 25 resulted in the highest reduction on day 90, and $H_{(17)}$ treatment which had the lowest value on day 25 resulted in the lowest reduction on day 90 (Table S15).

Soil NO_3^- was significantly different between treatments (N = 10, $\log_{10}$ transformation of data, F = 3.00, p-value = 0.023) as well as between the two time-points (N = 30, F = 10.16, p-value = 0.003), considering all samples from both day 25 and day 90. $P_{(1)}$ treatment had clearly the highest value (15.13 ±11.74), significantly different from *Control* (3.660 ±3.071) (T-value = 3.24, adjusted p-value = 0.028, Table S16). Also, contrary to soil NH_4^+, the total mean value of soil NO_3^- (9.63 ±7.76) on day 90 was significantly higher than that on day 25 (4.11 ±6.53) (T-value = 3.19, adjusted p-value = 0.003). Further analysis was conducted for each time-point separately. On day 25, soil NO_3^- were not significantly different between treatments (N = 5, $\log_{10}$ transformation of data, F = 2.18, p-value = 0.138). However, $P_{(1)}$ treatment had clearly the highest value (13.42 ±9.68) from all other treatments, while NO_3^- were not detected in *W* treatment (Table S16). Spearman correlations showed that soil NO_3^- on day 25 was significantly positively correlated with initial residue N (N = 5, ρ = 0.732, p-value < 0.001), and significantly negatively correlated with initial residue C:N ratio (N = 5, ρ = -0.728, p-value < 0.001), C (N = 5, ρ = -0.480, p-value = 0.015), and NDF content (N = 5, ρ = -0.701, p-value < 0.001) (Table S17). Likewise, there were no significant differences between treatments on day 90 (N = 5, Kruskal-Wallis test, H-value =4.95, p-value = 0.423). Again, $P_{(1)}$ treatment had the highest value (16.85 ±14.45), but the other treatments had relatively high values as well, with the lowest value again in *W* treatment (6.268

±2.157). However, in contrast to day 25, on day 90 Spearman correlations did not show any significant correlations between soil NO_3^- and initial residue chemical characteristics (p-values > 0.05). Treatments with the highest values on day 25 ($P_{(1)}$ and $S_{(6)}$ treatments) had the lowest increase on day 90 (Table S16).

Effect on soil microbiology

A number of 24 compounds were considered in Phospholipid Fatty Acid analyses on soil samples from 0-5 cm depth 52 days after mulch application to evaluate decomposer microbes' groups (nmol g^{-1} of freeze dry soil). The groups were represented the following compounds: Gram+ bacteria by C14:0, iC15:0, a-C15:0, C15:0, i-C16:0, i-C17:0, C18:0, and C20:0, Gram- bacteria by 2-OH-C12:0, C14:1, C15:1, 2-OH-C14:0, and 3-OH-C14:0, Fungi by C16:0, C18:3ω6, C18:2ω6c, C18:2ω6t, C18:1ω9c, C18:3ω3, and C20:1ω9, Protozoa by C20:4ω6 and C20:3ω6, and Cyanobacteria by C20:5ω3, although in previous studies C18:2ω6c has also been detected in Cyanobacteria, and C18:1ω9c and C18:3ω3 in higher plants and green algae (Quideau et al., 2016; Buyer & Sasser, 2012; Amir et al., 2010; Zelles, 1999; Frostegård & Bååth, 1996 Vestal & White, 1989).

Total microbial biomass, defined as the sum of all selected compounds, was not significantly different between treatments (N = 5, F = 0.34, p-value = 0.882). $H_{(17)}$ treatment had the highest value, and all other treatments had lower values than *Control* (Table S18). The same pattern with $H_{(17)}$, *C,* and $P_{(1)}$ treatments having the highest values and *W,* $S_{(6)}$, and $B_{(12)}$ treatments the lowest was observed in each microbial group separately, but with no significant differences in all cases. Spearman correlations showed that total soil microbial biomass had significant negative correlation (N = 5, ρ = -0.531, p-value = 0.006) with total soil C, on day 52, while none of these two were directly significantly correlated with initial residue chemical characteristics. However, total soil C had significant positive correlation with soil C:N ratio (N = 5, ρ = 0.634, p-value = 0.001), on day 52, which was significantly positively correlated with both initial residue C:N ratio (N = 5, ρ = 0.418, p-value = 0.037) and initial residue ADF (cellulose and lignin) content (N = 5, ρ = 0.466, p-value = 0.019), and significantly negatively correlated with initial residue N content (N = 5, ρ = -0.414, p-value = 0.040) (Table S17).

The fungi: bacteria ratio was higher in *Control*, *W* and $H_{(17)}$ treatments (Tables S16, and S19). Differences between the different treatments in each microbial group were not significant (p-value > 0.05), although clearly $H_{(17)}$ treatment had the highest values followed by *Control* and $P_{(1)}$ treatment, and $B_{(12)}$ treatment the lowest value, in most groups (Fig. 3, Tables 3, and S19). Spearman correlations showed that fungi: bacteria ratio was significantly positively correlated with both the initial residue NDF content (N = 5, ρ = 0.452, p-value = 0.023) and the initial residue lignin content (N = 5, ρ = 0.488, p-value = 0.013) (Table S17).

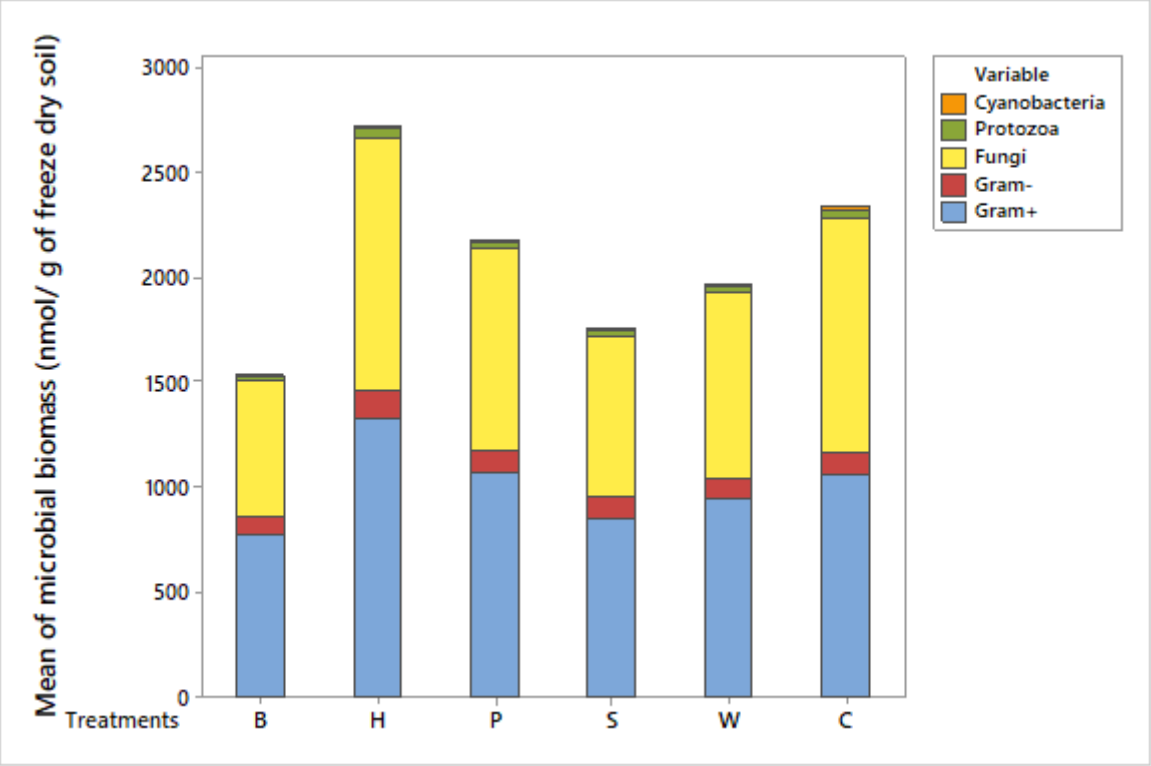

Fig. 3 Mass of different microbial groups (G+ = gram positive bacteria, G- = gram negative bacteria, F = fungi, P = protozoa, and C = Cyanobacteria), expressed in phospholipid fatty acids (PLFA) (nmol g^{-1} of freeze dry soil), for the different treatments (*B, Control (C), H, P, S, and W*) from soil samples at 0-5 cm depth, on day 52 after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*

A Principal Component Analysis (PCA) was conducted to detect differences between treatments including all microbial groups (Gram+, Gram-, Fungi, Protozoa, and Cyanobacteria) simultaneously.

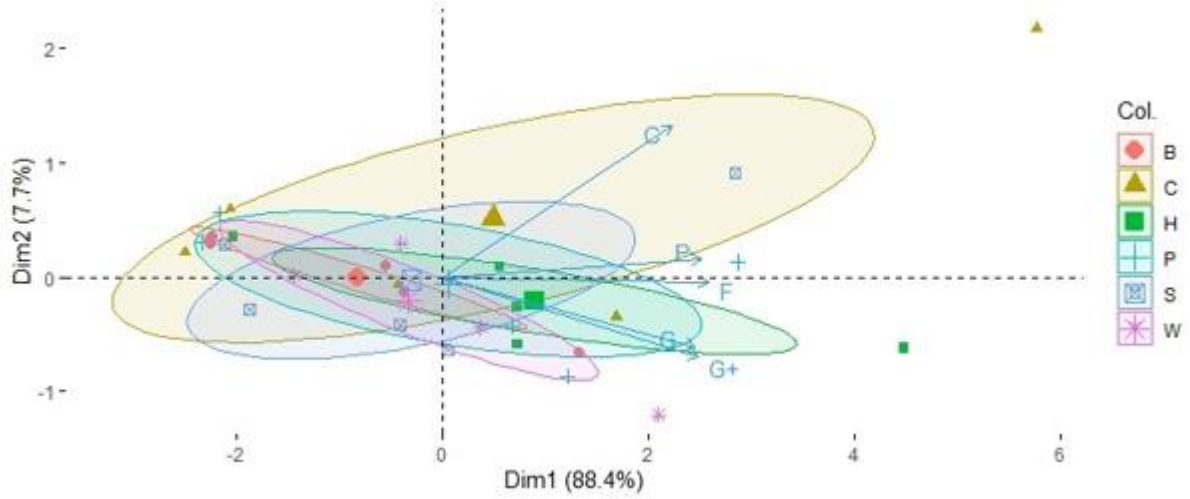

Fig. 4 Biplot of PCA for the different treatments (*B, Control (C), H, P, S,* and *W*) with microbial groups (G+ = Gram positive bacteria, G- = Gram negative bacteria, F = fungi, P = protozoa, and C = Cyanobacteria) as variables. Soil samples were collected from 0-5 cm depth, on day 52 after mulch application. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Large points depict mean values, while ellipses depict confidence intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1 normalized score, and left axis represents Dim2 normalized score

Biplot of PCA in Fig. 4 shows that all treatments were overlapped. Considering the place of the mean values in the graph we can see that, *Control* demonstrated positive relation with Cyanobacteria, while $S_{(6)}$ treatment had negative relations with Cyanobacteria and Protozoa. *W,* and $B_{(12)}$ treatments had negative relations with all microbial groups, but mostly with Gram+ and Gram- bacteria. $H_{(17)}$ treatment had strong positive relation mostly with Gram+ and Gram- bacteria, but also with fungi and Protozoa. $P_{(1)}$ treatment apparently had no relation with all microbial groups.

Effect on wheat plants

There were no significant differences in protein (N = 5, F = 0.29, p-value = 0.914), in C (N = 5, F = 0.54, p-value = 0.748), and in N content of seeds (N = 5, F = 0.29, p-value = 0.914) (Table S20). Dry mass of 1000 seeds was not significantly different between treatments (N = 5, F = 2.47, p-value = 0.061). *W* treatment had the highest value (39.81 ±5.58) and $P_{(1)}$ treatment the lowest (33.836 ±1.831) (Fig. S4, Table S21). Ear length (g) was significantly different between treatments (N = 75, F = 14.06, p-value < 0.001). *Control* (10.096 ±1.143) and *W* treatment (10.133 ±1.134) had the lowest values, significantly different from all other treatments (Fig. 5, Tables S21, and S22).

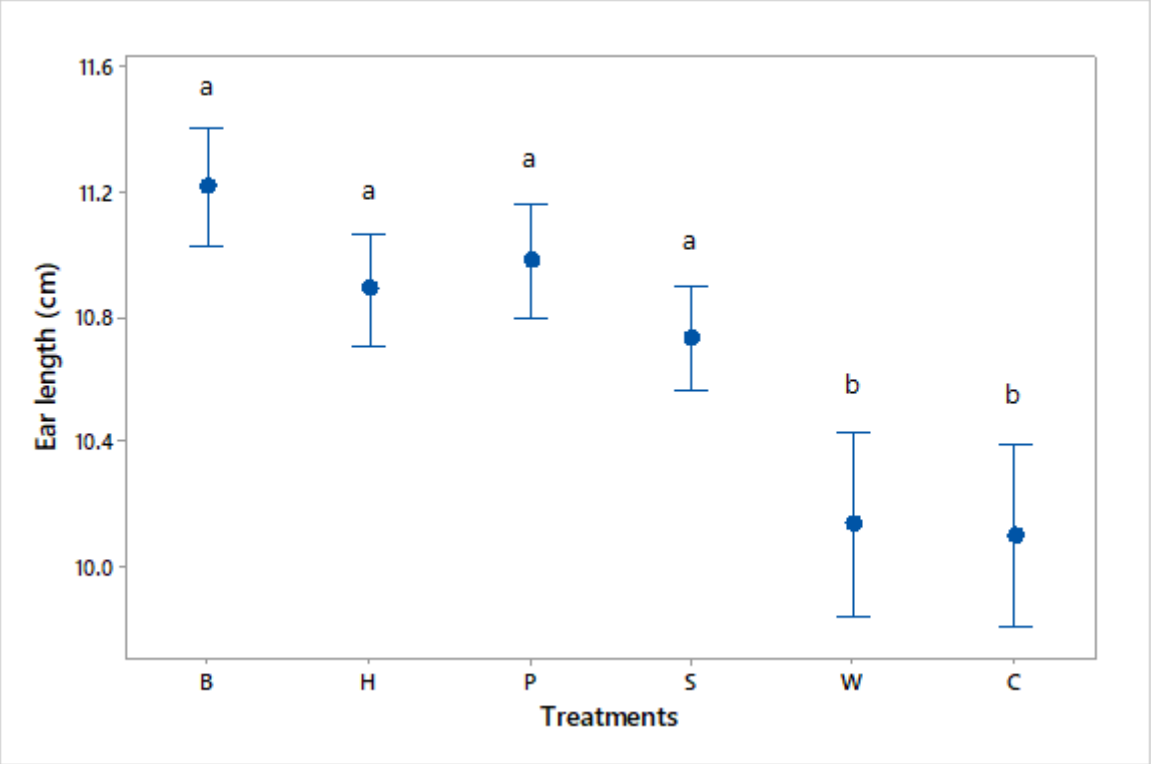

Fig. 5 Interval plot of ear length (cm) of the different treatments (*B, C, H, P, S*, and *W*), at harvest time (N = 75). Means and bars of one standard error from the mean are depicted. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. *Control (C)* treatment was with no residues. Means that do not share a common letter are significantly different (p-value < 0.05)

Moreover, *W* treatment (2.7286 ±0.6624) followed by $P_{(1)}$ treatment (2.739 ±0.887) and *Control* (2.7629 ±0.7738) had the lowest ear (with seeds) dry mass, although not significantly different from the other treatments (N = 75, Kruskal-Wallis test, H-value = 7.57, p-value = 0.182) (Table S21).

Spearman correlation revealed significant influences between variables related with residue chemical composition, and with seeds and ears (Table S23). Initial residue C significantly negatively affected both ear length (positively) dry mass of 1000 seeds (negatively) (Table S23), but it was not correlated significantly with seed C content (ρ = -0.087, p-value = 0.679). Furthermore, initial C residue was not correlated significantly with ear dry mass with seeds (ρ = -0.068, p-value = 0.748), although the later was significantly positively correlated with dry mass of 1000 seeds. Likewise, initial residue lignin significantly affected both ear length (negatively) and dry mass of 1000 seeds (positively) (Table S23).

Regression analyses showed significant linear regression (F = 13.85, p-value = 0.001) between ear length and initial residue C (r^2 = 37.59%), significant linear regression (F = 11.03, p-value = 0.003) between dry mass of 1000 seeds and initial residue C (r^2 = 32.41%), significant linear regression (F = 11.63, p-value = 0.002) between ear length and initial residue lignin (r^2 = 33.58%), and significant linear

regression (F = 9.63, p-value = 0.005) between dry mass of 1000 seeds and initial residue C (r^2 = 29.52%). The regression equations were:

Ear length = 22.67 - 0.263 initial residue C (1)

Dry mass of 1000 seeds = -37.9 + 1.631 initial residue C (2)

Ear length = 11.23 - 0.0615 initial residue lignin (3)

Dry mass of 1000 seeds = 33.06 + 0.385 initial lignin (4)

Differences between treatments considering a number of variables related with wheat plants simultaneously were also tested in a Principal Component Analysis.

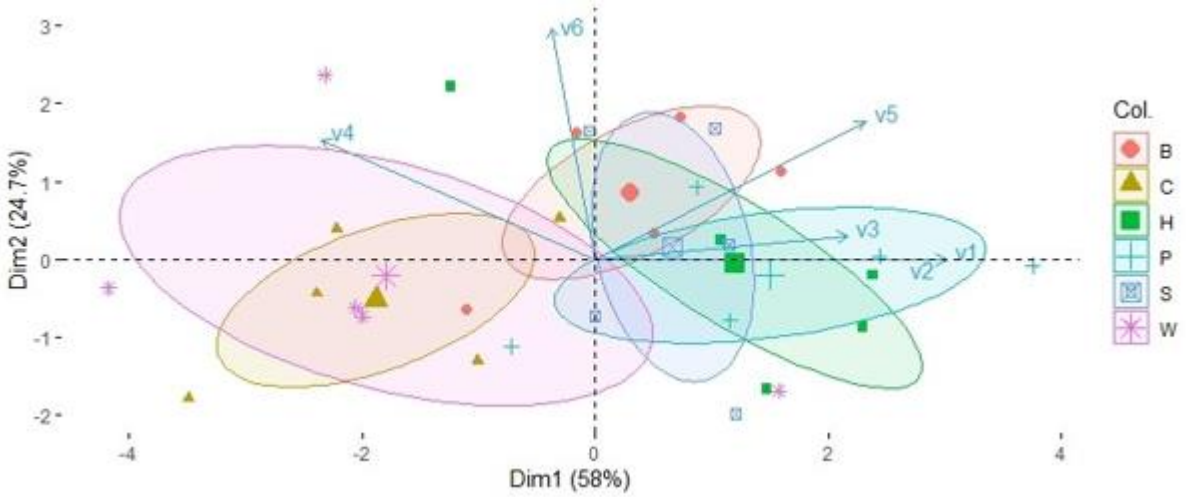

Fig. 6 Biplot of PCA for the different treatments (*B, Control (C), H, P, S,* and *W*) with variables related with wheat plants (v1 = % N of seeds, v2 = % protein of seeds, v3 = % C of seeds, v4 = dry mass of 1000 seeds, v5 = length of ears, v6 = dry mass of ears with seeds). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Large points depict mean values, while ellipses depict confidence intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1 normalized score, and left axis represents Dim2 normalized score

Biplot of PCA in Fig. 6 shows that three distinctive groups were formed in the graph by the different treatments. The first was formed by *Control* and *W* treatment, the second by $B_{(12)}$ and the third by $P_{(1)}$ treatments. Considering the place of the mean values in the graph, we see that $H_{(17)}$, $P_{(1)}$, and $S_{(6)}$ treatments were positively related with seed N, protein, and C content (v1, v2, and v3 variables), and negatively related with dry mass of 1000 seeds (v4). $B_{(12)}$ treatment was positively related with ear length, and ear dry mass (v5 and v6). *Control* and W were negatively related with seed N, protein, and C content (v1, v2, and v3 variables), and with ear length (v5), while *W* treatment was positively related with dry mass of 1000 seeds (v4).

Discussion

Initial and final residue quality

A residue C:N ratio of 24 is generally considered the threshold between N mineralization and immobilization, and net N mineralization is expected in values ≤ 24 during decomposition of residues, because the C:N ratio of soil microorganisms is around 8:1 and they can assimilate about 1:3 of the C they metabolize (Brust, 2019; Hadas et al., 2004). Nevertheless, this ratio could be higher under certain conditions as it is often in agroecosystems. Mesofauna for example such as nematodes, predatory on bacteria, contain less N than bacteria. Therefore, feeding by bacteria they release N to the soil, increasing thus optimal residue C:N ratio for N mineralization (Frouz, 2018). In our filed experiment *W* treatment had very high C:N ratio (50.865 ±1.321), $H_{(17)}$ and $B_{(12)}$ treatments had C:N ratio more than 30, $S_{(6)}$ and $P_{(1)}$ treatments less than 25 (Table S2). Therefore, W treatment (C:N>40) was expected to cause initial N immobilization (Brust, 2019). Decomposition of $H_{(17)}$ and $B_{(12)}$ treatments could be either ideal or lead to initial short-term N immobilization, depending on soil faunal composition (Frouz et al., 2015). Decomposition of $S_{(6)}$ and $P_{(1)}$ treatments were expected to drive to net N mineralization (Hadas et al., 2004). Previous experiments have showed that not only C:N ratio but also other characteristics such as N/P ratio, cellulose, lignin, and polyphenol content could highly influence decomposition rate (Zhonglu *et al.*, 2015; Austin and Ballaré, 2010; Cattanio et al., 2008).

The NDF content of residues could be used as a measure of initial recalcitrance (the higher the NDF the higher the recalcitrance) because it includes both the intermediately decomposable fractions hemicellulose and cellulose, and the slowly decomposable fraction lignin (Hadas *et al.*, 2004), but also the lignin content alone should be considered (Fog, 1988). Treatments were significantly different in their initial NDF and ADF content but not in lignin, except for *W* treatment (Table 1). Moreover, there were significant positive correlations between NDF and ADF, and between ADF and lignin (Table S7).

The final C:N ratios of $H_{(17)}$, $B_{(12)}$, $S_{(6)}$, and $P_{(1)}$ treatments had approximated each other, so the differences between them were not significant, but still *W* was significantly higher than all other treatments except *Control*. This proximity is justifiable as a decrease in residue C:N ratio (Akratos *et al.,* 2017) and an increase in lignin content is expected due to decomposition process (Frouz, 2018). The final recalcitrance, expressed by NDF was consistently higher in *W* and $H_{(17)}$ treatments and lower in $B_{(12)}$ and $P_{(1)}$ treatments, while the considerable increase of recalcitrance of the $S_{(6)}$ treatment was obviously linked with its high initial C content. Contrary, the order of treatments in lignin content was quite reversed. *W* treatment initially had the highest value, significantly different from all others, but displayed the lowest value in the end, although differences between treatments in final lignin content were not significant (Tables 1 and S12). Moreover, $B_{(12)}$ and $H_{(17)}$ treatments had higher values of final lignin than $P_{(1)}$ and $S_{(6)}$ treatments, opposite to initial lignin content. In previous research it was shown that the higher the C:N ratio of residues the higher the amount of lignin that was decomposed (Fog,

1988). In our experiment this was apparent only for residues with very high C:N ratio (*W* treatment), while it was not obvious at the other treatments with initial C:N ratio less than 40. However, differences between treatments in the final C:N ratio and the NDF content indicate that diversity or quality of residues affects their final quality at the end of the growing season. These observations lead to the inference that all C, N (or C:N ratio), NDF, and ADL should be considered in the interpretation of results concerning residue decomposition. The remaining residue biomass at the end of the growing season in combination with the wheat crop residues after harvest produced mulch of higher quality (C:N ratios among about 19 and 27) in comparison with the wheat crop residues alone (about 33), as predicted in our hypothesis (table S8). In addition, not only the C:N ratios were improved but also the lignin content of these residue mixes was not significantly different in all treatments including Control (wheat crop residues) (table S12). Moreover, the partly decomposed residues bear an increased amount of decomposer microbial biomass capable to decompose the more recalcitrant substances of the crop residues. This is very important for the nutrition of the successive crop.

Soil chemistry

Table 2 Classification of treatments (*B, Control (C), H, P, S,* and *W*) in descending order according to mean values in different soil properties. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Bulk soil represents soil conditions prior to mulch application. Samples were received 25, 52, and 90 days after mulch application (d25, d52, and d90 respectively). Treatments that do not share a common letter are significantly different (p-value < 0.05)

Properties	**Treatment order**	**Properties**	**Treatment order**
Soil solution pH (d52)	*Bulk soil>C>W>B>H>S>P*	Soil NO_3^- (d25)	*P>S>B>H>C>W*
Total % soil N (d52)	*P>C>H>W>B>S>Bulk soil*	Soil NO_3^- (d90)	*P>S>H>B>C>W*
Total % soil C (d52)	*C>H>W>B>S>P>Bulk soil*	Soil NO_3^- (d25 & d90)	$P^a>S^{ab}>H^{ab}>B^{ab}>W^{ab}>C^b$
Soil NH_4^+ (d25)	*P>C>W>B>S>H*		
Soil NH_4^+ (d90)	*W>P>H>C>S>B*		
Soil NH_4^+ (d25 & d90)	*P>W>C>H>B>S*		

Total soil N, and C at 0-5 cm depth, on day 52 was increased in comparison to the start of the experiment (bulk soil), in all treatments, although not significantly (Table 2). $P_{(1)}$ treatment (the richest in N residues) had the highest value in total soil N followed by *Control*, while *Control* had the highest value, and $P_{(1)}$ treatment the lowest in total soil C. This indicates an increased although slow contribution of high-quality residues in N release in comparison to residues of lower quality, but also no particular contribution of treatments with residues in C sequestration in comparison to *Control*. By the rest treatments, those with residues of lower quality (*W*, and $H_{(17)}$ treatments) had higher total soil

C than those of higher quality (Tables 2, and S14). Therefore, there was an indication that residue chemical composition had an impact on soil nutrient stoichiometry at advanced stages of decomposition in line with previous research (Piaszczyk *et al.*, 2019). However, in the particular field conditions this impact was week and possibly overshadowed by pre-existing and parallel biochemical processes in the soil.

The influence of residue chemical composition on N dynamics was more obvious in soil NH_4^+ and NO_3^- content (Table 2). These contents are more representative of the soil potential of the direct N availability, while total soil N concerns both the available N and the N linked with the soil organic matter. Soil NH_4^+ at 30-45 cm depth was significantly higher on day 25 than on day 90, while the opposite was true for soil NO_3^- (Tables S16, and S18). Soil NH_4^+ at this depth possibly can arise either by leaching of NH_4^+ in organic form or by denitrification under limited oxygen conditions (Ambus et al., 2001). Soil NO_3^- can result by nitrification of NH_4^+ released from decomposition of organic material, in well oxidized soil, and are easily subject to leaching. Their production is favoured by residues rich in N, like legumes (White et al., 2017). Increased NO_3^- and decreased NH_4^+ on day 90 in comparison to day 25 was the result of nitrification process, but it may be an indication of deceleration of decomposition process due to the consumption of the easily decomposable material of residues, followed by decomposition of more recalcitrant material in a slower rate. Normally (no flooding conditions), NH_4^+ are supposed to decline over time (Castro & Whalen, 2016). Residues of higher quality ($P_{(1)}$ treatment) had the highest values on both soil NH_4^+ and NO_3^- considering samples from both day 25 and day 90 (Tables 2, S15, and S18). Treatments of residues with high N content ($P_{(1)}$, and $S_{(6)}$ treatments) had the highest values in soil NO_3^- followed by treatments with low N, and high recalcitrance but low lignin content ($H_{(17)}$, and $B_{(12)}$ treatments), while *Control* and *W* treatment had the lowest values. This pattern was the same on both day 25 and day 90 and the results were significantly different between $P_{(1)}$ treatment and *Control* considering both time-points together (Tables 2, and S18). The influence of residue quality on soil N availability was further confirmed by the significant Spearman correlations between soil NO_3^- and initial residue chemical characteristics (C:N ratio, C, N, and NDF) on day 25 (Table S17). Truong and Marschner (2018) also observed enhanced available N from decomposition of litter rich in N, in comparison to material with very high C:N ratio (> 40). Therefore, our hypothesis that residue diversity or quality affects N mineralization was confirmed, and this was due to differences in chemical composition of the different diverse residue types.

Cultivation of wheat resulted in reduction of soil solution pH at 0-5 cm depth in July (day 52) in comparison to the first pH measurement in May, although not significantly different between treatments. Treatments of high quality ($P_{(1)}$ and $S_{(6)}$) had the lowest mean values. This was congruous with previous research concluded that green manure can drop soil solution pH in a short- or in a long-term by producing organic acids during their decomposition (Singh & S. Khind, 1992). *Control* and *W*

had the highest pH values which were justified by lower nitrification rates observed in samples from both day 25 and day 90, because NO_3^- can drop soil solution pH (Fog, 1988) (Table S16).

Soil microbiology

Soil solution pH should be examined in combination with soil microbial activity. It is well known that fungi are favoured by low soil solution pH, and bacteria the opposite (Barreiro et al., 2016). In our experiment the values of soil solution pH between treatments were very close and differences were insignificant. Nevertheless, *Control* and treatments with lower-quality residues (*W* and $H_{(17)}$ treatment) demonstrated higher fungi: bacteria ratio, while the opposite was true for residues of higher quality, on day 52, in consistence with previous research (Frasier *et al.*, 2016). The significant positive correlations that were observed between the fungi: bacteria ratio and with the initial residue NDF or the initial residue lignin (recalcitrant substances) were strong indications that diversity or quality of residues can alter soil environment affecting soil microbial biomass. A high fungal: bacterial ratio is linked with residues of higher C:N ratio and higher recalcitrance (Barreiro *et al.*, 2016). However, we cannot assume that our hypothesis, that soil microbial biomass at a later stage of decomposition is affected by residue diversity or quality, was fully confirmed because differences were not significant.

Soil microbial diversity is affected by aboveground diversity, but it is influenced by temporal, spatial and hierarchical factors (Lukac, 2017). Differences between treatments in soil microbial biomass, at 0-5 cm depth, on day 52, were not significantly different in all cases of microbial groups. Lack of significant differences in soil respiration activity between litter of low and high C:N ratio, 48 days after litter addition, was also reported in another experiment (Truong & Marschner, 2018). However, a consistent influence pattern of residue diversity was observed. $H_{(17)}$ treatment had the highest values in soil microbial biomass of all microbial groups, followed by *Control*, $P_{(1)}$, and *W* treatments, while $S_{(6)}$, and $B_{(12)}$ treatments had the lowest (Table 3). Probably, $H_{(17)}$ treatment, with initial C:N ratio = 33.5, ideally provided soil microbes with both readily available labile C and N, while in other treatments either C ($P_{(1)}$ treatment) or N (*W* and $S_{(6)}$ treatments) were limited factors for soil microbes. This assumption is reinforced by the fact that mulch residue decomposition is driven largely by fungi rather than bacteria due to reduced direct contact with soil (Barreiro *et al.*, 2016; van der Heijden *et al.*, 2008). Fungi are characterized by higher C:N ratio than bacteria and also can transfer nutrients through their hyphae so the ideal substrate C:N ratio could be a little higher (Liang *et al.*, 2017; Hattenschwiler *et al.*, 2005) than that which is considered optimal (Frouz *et al.*, 2015). However, the behaviour of $B_{(12)}$ treatment which had quite similar values of initial residue properties with $H_{(17)}$ treatment cannot be directly explained by this assumption. An explanation could be hidden in Nitrogen/ Phosphorus ratio (Zhonglu et al., 2015) or in polyphenolic content of initial residues which were not considered in the experiment. The gradual increase of phenolic and aromatic substances in residues causes deceleration of decomposition rate (Xu et al., 2017). Evenly, it could be attributed to the special composition of

plant species (diversity) participated in plant residue mixtures subjected to microbial enzymatic specificity during decomposition (Fontaine *et al.,* 2003) or provided soil microbes with essential nutrients like P, Fe, and Zn (Li *et al.*, 2014).

Table 3 Classification of treatments (*B, Control (C), H, P, S,* and *W*) in descending order according to mean values of soil content in different microbial group biomass, 52 days after mulch application. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*

Microbial groups	**Treatments' order**	**Microbial groups**	**Treatments' order**
Total microbial biomass	*H>C>P>W>S>B*	Protozoa	*H>C>P>W>S>B*
Gram+	*H>P>C>W>S>B*	Cyanobacteria	*C>H>P>B>S>W*
Gram-	*H>C>P>S>W>B*	Bacteria (G+ & G-)	*H>P>C>W>S>B*
Fungi	*H>C>P>W>S>B*	Fungi: Bacteria	*C>W>H>P>S>B*

It is remarkable, *Control* followed $H_{(17)}$ treatment which had the highest values of soil microbial biomass in almost all microbial groups. This may be an indication of soil nutrient limitations due to imbalanced release of nutrients from plant residues, resulting in increased microbial competition in all other treatments. It has been shown that decomposition rate and consequently residue mass loss is decelerating over time (Tibbett *et al.*, 2004). Therefore, it is possible, after an initial acceleration of decomposition rate where easily decomposable material was consumed by soil microbes, the decomposition of more recalcitrant material resulted in those nutrient limitations, evidenced on day 52 (Xu *et al.*, 2017; Reichert *et al.*, 2015). Furthermore, the treatments' order regarding their effect on each microbial group followed largely the same pattern on day 52, but this was only an indication of the effect of residue diversity on microbial biomass, because significant differences were not observed. However, the total microbial biomass was significantly negatively correlated with total soil C, which was significantly positively correlated with soil C:N ratio, on day 52, which was significantly positively correlated with both initial residue C:N ratio and ADF content, and significantly negatively correlated with initial residue N content. These correlations provide a strong indication that, although a shift in decomposition rate from high- to low-quality residues apparently was in progress, the total microbial biomass on day 52 was still favoured by residues with a relatively low initial C:N ratio and low ADF content. Therefore, there were indications that our hypothesis that residue diversity or quality affect soil microbial biomass was true, but this was not fully supported. In contrast significant effect of residue diversity was observed in microbial diversity in other research (Fontaine et al., 2003; Wu et al., 1993). We could suggest, in accordance with Calderon et al. (2016), longer time is necessary to manifest such a relation. Continuous applications of long-residue mulch on agroecosystems may provide environmental conditions approximating natural ecosystems favouring the development of more diverse microbial communities (Santonja *et al.*, 2017). Natural ecosystems were found to

increase easily decomposable soil C compounds and microbial enzymatic activity in comparison with agroecosystems (Blonska *et al.*, 2020).

Wheat plant quality (chemical composition and morphology)

Table 4 Classification of treatments (*B, Control (C), H, P, S,* and *W*) in descending order according to mean values of different properties related with yield (seed C, N, and protein % content) and quality (dry mass of 1000 seeds (g), ear length (cm), and ear dry mass (g)) of grains of wheat plants, 87 days after mulch application. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Treatments that do not share a common letter are significantly different (p-value < 0.05)

Properties	**Treatments' order**	**Properties**	**Treatments' order**
Seed C	*P>C>B>W>H>S*	Dry mass of 1000 seeds (g)	*W>C>S>B>H>P*
Seed N	*B>W>S>C>H>P*	Ear length (cm)	$B^a>P^a>H^a>S^a>W^b>C^b$
Seed protein	*B>W>S>C>H>P*	Ear dry mass (g)	*B>S>H>C>P>W*

The morphology rather than nutritional value of seeds seems to be more obviously influenced by the residue diversity or quality (Table 4). Mulch significantly contributed to ear length increase. *Control* had the lowest value followed by *W* treatment (residues of very low quality), significantly different from all other treatments. Initial C content of residues was a significant negative influential parameter as it was confirmed by regression analysis. Initial residue C could explain r^2 = 37.59% of the ear length variation. Treatments $B_{(12)}$ and $P_{(1)}$ which had the lowest initial C content resulted in longer ear length than treatments $H_{(17)}$ and $S_{(6)}$. In addition, initial residue lignin was significantly negatively affected ear length, and regression analysis showed that it could explain r^2 = 33.58% of the variation. Moreover, the influence of initial residue N, C:N ratio, and NDF content on ear length was not significant (p-value > 0.05). Thus, other chemical parameters, like initial P or K or phenolic content of residues or soil physical properties like soil temperature or soil moisture (affected by residue morphology) could possibly justify the unexplained variation of the ear length.

Likewise ear length, Spearman correlation showed that both initial residue C and lignin contents significantly affected the dry mass of 1000 seeds. Regression analysis showed that initial residue C could explain the r^2 = 32.41% and initial residue lignin the r^2 = 29.52% of the dry mass of 1000 seeds variation. Apparently, treatments with shorter ear length had heavier seeds. Dry mass of 1000 seeds was higher in *W* and *Control* treatments, although not significantly different from other treatments. However, ear length was not significantly correlated with dry mass of 1000 seeds (p-value < 0.05). Nevertheless, the influence of residue chemical composition in ear length was stronger than that in dry mass of 1000 seeds, as it was confirmed by higher absolute values of Spearman correlation coefficients (ρ) and lower p-values (Table S23). This was also indicated by the fact that $B_{(12)}$ treatment

(which had the highest value of ear length) had the highest value of ear dry mass with seeds, and *W* treatment (which had the highest value of dry mass of 1000 seeds) the lowest, although not significantly different.

Furthermore, according to Table S23, seed protein was highly positively correlated not only with seed N, but also with seed C, and seed C:N ratio. This implies that an increase either in seed N or in seed C was followed by an increase in seed protein content. However, neither seed N nor seed C were significantly correlated either with initial residue N or with initial residue C. Therefore, there was no clear evidence that initial residue chemical composition affected seed protein content. $P_{(1)}$ treatment which had the highest initial N content resulted in the lowest N, and protein content of seeds. Accordingly, $S_{(6)}$, $H_{(17)}$, and *W* treatments which had the highest initial C in residues resulted in the lowest values of seed C.

The results in PCA (Fig. 3) where distinctive groups were formed, confirmed that residue diversity or quality can significantly affect crop quality, in line with our hypothesis. Therefore, it seems that in the particular field conditions the initial residues chemical composition influenced mostly the wheat plant growth and consequently the wheat plant morphology rather than the seed nutritional characteristics.

Conclusions

The remaining residue biomass in mixtures with crop residues at harvest time produced mulch of higher quality than the crop residues alone. It seems that all treatments resulted in enrichment of total soil C and N, on day 52 (52 days after mulch application), in comparison to the bulk soil, although differences were not significant. High-quality residues might contribute to increase of total soil N, and residues of lower quality to increase of total soil C, but the influence of residue diversity on total soil C and N was not clear. Soil NO_3^- content at 30-45 cm depth was increased on day 90 in comparison to day 25, while the opposite was true for soil NH_4^+. Soil NO_3^- was increased in all treatments with residues in comparison to *Control*, significantly in residues of higher quality, considering samples from both day 90 and day 25. Soil NO_3^- on day 25 was favoured in residues of higher quality than those of lower quality, because it was significantly positively correlated with initial residue N, and significantly negatively correlated with initial residue C:N, C, and NDF content. The influence of residue diversity or residue quality on soil NH_4^+ was not clear, at both d25 and day 90. Soil solution pH was reduced in all treatments on day 52 in comparison to the start of the experiment, as a result of wheat cultivation. The reduction was higher in treatments with residues, and among these in treatments of higher quality, but in all cases the differences were not significant. There were indications that residues of lower quality resulted in higher fungi: bacteria ratio than those of higher quality, at 0-5 cm depth, on day 52. A narrow residue C:N ratio of about 33 was seemed to be optimal to increase soil microbial biomass, at 0-5 cm depth, on day 52, while probably with residues of higher or lower C:N ratios either N or C, respectively, became a limiting factor, although differences were not significant. There were

indications that soil microbial biomass on day 52 was favoured by residues of higher quality than those of lower quality. Residue chemical composition influenced wheat crop plant morphology rather than seed nutritional value. Both, residue C and residue lignin contents were significantly negatively correlated with wheat ear length and significantly positively with wheat seed weight. However, there was not an apparent effect of residue chemical composition on seed C, N, or protein content. Generally, mulch of whole-plant diverse residues in field conditions can affect N mineralization and crop quality, but any influence on soil microbial biomass at a later stage of decomposition was not apparent. The remaining residue biomass in combination with crop residues at harvest time can produce mulch of higher quality than that of the crop residues alone, in benefit of the successive crop. Further research is needed with continuous applications of whole-plant diverse residues in field conditions to determine notable functional characteristics, optimal amounts, and sufficient number of applications to maximize benefits.

Declarations

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Availability of data and material: Not applicable.

Code availability: Not applicable.

Ethics approval: Not applicable.

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Fig. S1 Experimental plots soon after mulch application

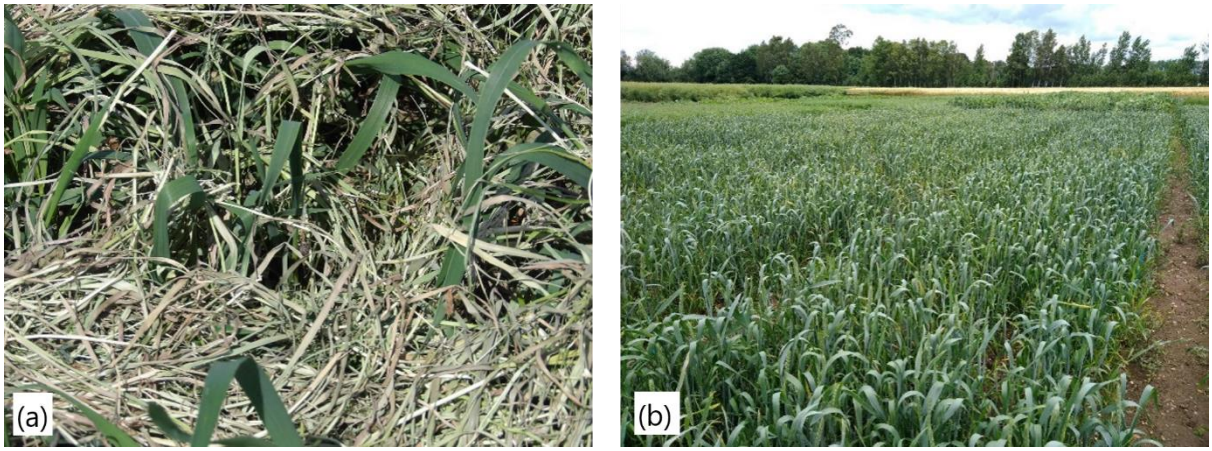

Fig. S2 From left to right a) Crop plants emerging among plant residues, b) Experimental plots at the growing stage of GS40 to GS49 (booting)

Fig. S3 a) Crop plants growing among plant residues at the growing stage of GS60 to GS65 (anthesis), b) Experimental plots after harvest (the already decomposed residues were mixed with crop plant residues)

Table S1 Diverse forage mixture species selection list (P: Perennial Ryegrass; S: Smart Grass; B: Biomix; H: Herbal)

Species	Latin	P	S	B	H
Perennial Ryegrass	Lolium perenne L.	✓	✓	✓	✓
Timothy	Phleum pratense L.		✓	✓	✓
Cocksfoot	Dactylis glomerata L.			✓	✓
Festulolium	-			✓	✓
Tall Fescue	Festuca arundinacea Schreb.				✓
Meadow Fescue	Festuca pratensis Huds.			✓	✓
Red Clover	Trifolium pratense L.		✓	✓	✓
White Clover	Trifolium repens L.		✓	✓	✓
Alsike Clover	Trifolium hybridum L.			✓	✓
Sweet Clover	Melilotus spp.				✓
Black Medick	Medicago lupulina L.			✓	
Lucerne	Medicago sativa L.			✓	
Sainfoin	Onobrychis spp.				✓
Birdsfoot Trefoil	Lotus corniculatus L.				✓
Plantain	Plantago lanceolata L.		✓	✓	✓
Chicory	Cichorium intybus L.		✓	✓	✓
Yarrow	Achillea millefolium L.				✓
Burnet	Sanguisorba minor Scop.				✓
Sheep's Parsley	Petroselenium crispum Mill.				✓

Table S2 Initial C:N ratio, % C, % N, and % protein content of the different residue types (N = 6). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means that do not share a common letter are significantly different ($p<0.05$).

	C:N ratio		C		N		protein	
Types	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	30.915^{c}	0.404	44.463^{c}	0.059	1.4384^{c}	0.0174	8.1989^{c}	0.0993
H	33.523^{b}	0.357	44.867^{b}	0.041	1.3385^{d}	0.0134	7.6294^{d}	0.0764
P	12.849^{e}	0.0570	43.942^{d}	0.062	3.4199^{a}	0.0155	19.4930^{a}	0.0883
S	22.733^{d}	0.329	44.923^{b}	0.030	1.9765^{b}	0.0285	11.2660^{b}	0.1630
W	50.865^{a}	1.321	47.579^{a}	0.179	0.9358^{e}	0.0238	5.3346^{e}	0.1360

Table S3 Tukey's post-hoc test for differences between types in initial C:N ratio, % C, and % N of residues (N = 6). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

C:N ratio	C	N

Difference of Levels	T-Value	Adjusted P-Value	Difference of Levels	T-Value	Adjusted P-Value	Difference of Levels	T-Value	Adjusted P-Value
H - B	9.07	<0.001	H - B	7.62	<0.001	H - B	-8.43	<0.001
P - B	-98.33	<0.001	P - B	-9.83	<0.001	P - B	167.25	<0.001
S - B	-34.44	<0.001	S - B	8.67	<0.001	S - B	45.42	<0.001
W - B	55.75	<0.001	W - B	58.77	<0.001	W - B	-42.41	<0.001
P - H	-107.40	<0.001	P - H	-17.45	<0.001	P - H	175.68	<0.001
S - H	-43.51	<0.001	S - H	1.05	0.830	S - H	53.85	<0.001
W - H	46.67	<0.001	W - H	51.15	<0.001	W - H	-33.98	<0.001
S - P	63.89	<0.001	S - P	18.50	<0.001	S - P	-121.83	<0.001
W - P	154.07	<0.001	W - P	68.61	<0.001	W - P	-209.66	<0.001
W - S	90.18	<0.001	W - S	50.10	<0.001	W - S	-87.83	<0.001

Table S4 Pearson correlation r values between initial C:N ratio, % C, and % N content of residue types (N = 6). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values are in parentheses. Correlations with p-values <0.05 are significant

All types	C:N ratio	C	***B* type**	C:N ratio	C
C	0.896 (<0.001)		C	0.746 (0.089)	
N	-0.899 (<0.001)	-0.680 (<0.001)	N	-0.997 (<0.001)	-0.690 (0.129)
***H* type**			***P* type**		
C	0.693 (0.127)		C	0.109 (0.837)	
N	-0.997 (<0.001)	-0.639 (0.172)	N	-0.949 (0.004)	0.209 (0.691)
***S* type**			***W* type**		
C	0.188 (0.722)		C	0.296 (0.569)	
N	-0.999 (<0.001)	-0.143 (0.787)	N	-0.988 (<0.001)	-0.146 (0.783)

Table S5 Initial NDF, ADF, ADL, hemicellulose, and cellulose (%) content of the different residue types (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin). Means that do not share a common letter are significantly different (p-value < 0.05).

	NDF		**ADF**		**ADL**	
Types	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	54.047[b]	0.410	29.912[b]	0.272	3.483[b]	1.173
H	54.652[b]	0.754	30.414[b]	0.350	3.437[b]	0.585
P	44.063[c]	0.308	23.023[c]	0.194	7.730[b]	6.550

S	44.283[c]	0.402	23.283[c]	0.229	6.090[b]	3.050
W	59.367[a]	0.983	42.331[a]	0.618	15.095[a]	0.743

	Hemicellulose		**Cellulose**	
Types	**Mean**	**StDev**	**Mean**	**StDev**
B	24.136[a]	0.187	26.428[a]	1.393
H	24.239[a]	0.482	26.976[a]	0.656
P	21.040[b]	0.249	15.290[b]	6.500
S	20.999[b]	0.295	17.190[b]	2.910
W	17.036[c]	0.537	27.236[a]	0.663

Table S6 Tukey's post-hoc test for differences between residue types in initial NDF, ADF, ADL, hemicellulose, and cellulose (%) content of residues (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin). P-values <0.05 indicate significant differences

NDF			**ADF**			**ADL**		
Difference of Levels	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
P - B	-25.22	< 0.001	P - B	-29.80	< 0.001	W - B	5.56	< 0.001
S - B	-24.67	< 0.001	S - B	-28.67	< 0.001	W - H	5.58	< 0.001
W - B	13.44	< 0.001	W - B	53.72	< 0.001	W - P	3.53	0.016
P - H	-26.75	< 0.001	P - H	-31.97	< 0.001	W - S	4.31	0.003
S - H	-26.20	< 0.001	S - H	-30.84	< 0.001			
W - H	11.91	< 0.001	W - H	51.55	< 0.001			
W - P	38.66	< 0.001	W - P	83.52	< 0.001			
W - S	38.11	< 0.001	W - S	82.40	< 0.001			
Hemicellulose			**Cellulose**					
Difference of Levels	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**			
P - B	-13.04	< 0.001	*P - B*	-5.38	< 0.001			
S - B	-13.21	< 0.001	*S - B*	-4.46	0.002			
W - B	-29.90	< 0.001	*P - H*	-5.65	< 0.001			
P - H	-13.47	< 0.001	*S - H*	-4.73	0.001			
S - H	-13.64	< 0.001	*W - P*	5.77	< 0.001			
W - H	-30.34	< 0.001	*W - S*	4.85	0.001			
W - P	-16.86	< 0.001						
W - S	-16.69	< 0.001						

Table S7 Pearson correlation r values between initial NDF, ADF, ADL, hemicellulose, and cellulose (%) content of residues including all types together (N = 25). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values are in parentheses. Correlations with p-values <0.05 are significant

	NDF	**ADF**	**ADL**	**Hemicellulose**
ADF	0.927			

	(<0.001)			
ADL	0.289	0.550		
	(0.161)	(0.004)		
Hemicellulose	-0.147	-0.507	-0.788	
	(0.484)	(0.010)	(<0.001)	
Cellulose	0.832	0.691	-0.223	0.090
	(<0.001)	(<0.001)	(0.284)	(0.670)

Table S8 Final C:N ratio, % C, % N, and % protein content of the different residue treatments (*B, Control (C), H, P, S,* and *W*) (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means that do not share a common letter are significantly different (p<0.05).

	C:N ratio		C		N		protein	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	24.75^{bc}	5.49	26.51	2.27	1.123	0.304	6.399	1.735
C	32.38^{ab}	2.97	29.43	4.66	0.916	0.181	5.224	1.034
H	26.82^{bc}	6.01	29.08	2.72	1.119	0.210	6.379	1.198
P	19.55^{c}	0.62	25.28	2.94	1.297	0.184	7.394	1.051
S	24.18^{bc}	5.21	29.37	1.29	1.291	0.443	7.360	2.530
W	36.03^{a}	5.33	29.97	5.43	0.838	0.163	4.780	0.933

Table S9 Tukey's post-hoc test for differences between treatments (*B, Control (C), H, P, S,* and *W*) in final C:N ratio of residues (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

Difference of Levels	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
W - B	3.82	0.010	*W - H*	3.12	0.047
P - C	-4.34	0.003	*W - P*	5.58	<0.001
S - C	-2.77	0.097	*W - S*	4.01	0.006

Table S10 Pearson correlation r values between final C:N ratio, % C, and % N content of residue types and *Control* (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values are in parentheses. Correlations with p-values <0.05 are significant

Control	C:N ratio	C	***B* type**	C:N ratio	C
C	-0.206		C	-0.466	
	(0.739}			(0.429)	
N	-0.582	0.913	N	-0.955	0.676
	(0.303)	(0.030)		(0.011)	(0.210)
***H* type**			***P* type**		

C	0.673 (0.214)		C	-0.803 (0.102)	
N	-0.927 (0.023)	-0.368 (0.542)	N	-0.875 (0.052)	0.991 (0.001)
***S* type**			***W* type**		
C	-0.814 (0.093)		C	0.435 (0.464)	
N	-1.000 (<0.001)	0.819 (0.090)	N	-0.352 (0.561)	0.688 (0.199)

Table S11 Final hemicellulose, and cellulose (%) content, and Tukey's post-hoc test for differences in % cellulose content of the different treatments (*B, Control (C), H, P, S,* and *W*) (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means that do not share a common letter are significantly different (p<0.05). P-values <0.05 indicate significant differences

	Hemicellulose		**Cellulose**		**Cellulose**		
Types	**Mean**	**StDev**	**Mean**	**StDev**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
B	13.968	1.206	16.15^{ab}	1.01	P-C	-3.10	0.050
C	15.166	1.859	19.21^{a}	2.98	P-H	-3.19	0.040
H	16.678	1.579	19.40^{a}	2.54	W-P	3.20	0.040
P	13.381	1.282	13.17^{b}	1.23			
S	15.673	1.796	18.18^{ab}	3.54			
W	13.790	2.900	19.41^{a}	5.16			

Table S12 Final NDF, ADF, and ADL (%) content of the different treatments (*B, Control (C), H, P, S,* and *W*) (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. NDF = Neutral Detergent Fiber (Hemicellulose, Cellulose and Lignin), ADF = Acid Detergent Fiber (Cellulose and Lignin), and ADL = Acid Detergent Lignin (Lignin). Means that do not share a common letter are significantly different (p<0.05).

	NDF		**ADF**		**ADL**	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	63.09^{ab}	3.26	49.12	2.26	32.96	2.75
C	63.04^{ab}	2.82	47.88	4.42	28.66	7.31
H	65.81^{a}	2.63	49.13	2.68	29.74	3.88
P	58.65^{b}	2.60	45.27	3.62	32.09	4.32
S	65.74^{a}	3.97	50.07	3.07	31.88	2.77
W	64.44^{ab}	5.51	50.65	5.52	31.23	8.20

Table S13 Pearson correlation r values between final NDF, ADF, ADL, hemicellulose, and cellulose (%) content of residues including all types and *Control* treatment together (N = 30). The residue types were:

Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values are in parentheses. Correlations with p-values <0.05 are significant

	NDF	ADF	ADL	Hemicellulose
ADF	0.868			
	(<0.001)			
ADL	0.317	0.699		
	(0.088)	(<0.001)		
Hemicellulose	0.368	-0.143	-0.676	
	(0.045)	(0.451)	(<0.001)	
Cellulose	0.480	0.088	-0.651	0.790
	(0.007)	(0.642)	(<0.001)	(<0.001)

Table S14 Soil solution pH, % total soil C, and % total soil N of the different treatments (*B, Control (C), H, P, S,* and *W*), from soil samples at 0-5 cm depth, 52 days after mulch application, when wheat plants were at growing stage GS75-80 (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Values from bulk soil are quoted for comparisons

	Soil solution pH		Total soil C		Total soil N	
Types	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	5.480	0.543	2.0767	0.1654	0.2421	0.0162
C	5.532	0.238	2.1513	0.1143	0.2455	0.0102
H	5.430	0.352	2.1494	0.1317	0.2452	0.0107
P	5.134	0.216	2.0413	0.0774	0.2481	0.0060
S	5.376	0.321	2.0609	0.0806	0.2408	0.0058
W	5.488	0.318	2.0852	0.1066	0.2426	0.0087
Bulk soil	5.592	0.371	2.0420	0.2270	0.2339	0.0245

Table S15 Soil NH_4^+ (mg kg^{-1} of oven dry soil) content of the different treatments (*B, Control (C), H, P, S,* and *W*), on day 25 and on day 90 after mulch application, and mean difference between the two time-points (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. *Control (C)* was with no residues.

	Day 25		Day 90		
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean diff.**
B	4.137	0.874	2.254	0.226	-1.883
C	4.322	0.405	2.363	0.203	-1.958
H	3.897	0.456	2.514	0.357	-1.383
P	4.472	0.995	2.571	0.680	-1.901
S	4.015	0.676	2.335	0.195	-1.679
W	4.259	0.303	2.576	0.417	-1.683

Table S16 Soil NO_3^- (mg kg^{-1} of oven dry soil) content of the different treatments (*B, Control (C), H, P, S,* and *W*), on day 25 and on day 90 after mulch application (N = 5), mean difference between the two time-periods, and soil NO_3^- (mg kg^{-1} of oven dry soil) content in combined samples from both day 25 and day 90 (N = 10). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Means that do not share a common letter are significantly different (p-value < 0.05).

	Day 25		Day 90			Both day 25 and day 90		
Treatments	Mean	StDev	Mean	StDev	Mean diff.	Mean	StDev	p-value of coefficients
B	1.88	3.36	7.34	2.76	5.46	4.61^{ab}	4.08	0.301
C	0.91	1.25	6.40	0.87	5.49	3.66^{b}	3.07	0.062
H	1.60	2.31	10.03	7.33	8.43	5.82^{ab}	6.78	0.498
P	13.42	9.68	16.85	14.45	3.43	15.13^{a}	11.74	0.004
S	6.84	5.63	10.86	7.78	4.02	8.85^{ab}	6.74	0.198
W	0.00	0.00	6.26	2.15	6.26	3.13^{ab}	3.60	

Table S17 Spearman correlations between variables related with soil microbial biomass (total microbial biomass, fungi: bacteria ratio) on day 52 (52 days after mulch application), variables related with soil chemical characteristics on day 52 (total soil C, soil C:N ratio) and on day 25 (soil NO_3^-), and variables related with initial residue chemical characteristics (C:N, C, N, NDF, ADF, and lignin) (N = 5). Correlations with p-value < 0.05 were statistically significant.

Sample 1	Sample 2	Spearman correlation coefficient (ρ)	p-value
Total microbial biomass (day 52)	Total soil C (day 52)	-0.531	0.006
Total soil C (day 52)	Soil C:N (day 52)	0.634	0.001
Soil C:N (day 52)	Initial residue C:N	0.418	0.037
Soil C:N (day 52)	Initial residue ADF	0.466	0.019
Soil C:N (day 52)	Initial residue N	-0.414	0.040
Fungi: bacteria	Initial residue NDF	0.452	0.023
Fungi: bacteria	Initial residue lignin	0.488	0.013
Soil NO_3^- (day 25)	Initial residue C:N	-0.728	<0.001
Soil NO_3^- (day 25)	Initial residue C	-0.480	0.015
Soil NO_3^- (day 25)	Initial residue N	0.732	<0.001
Soil NO_3^- (day 25)	Initial residue NDF	-0.701	<0.001

Table S18 Phospholipid fatty acid (PLFA) compounds (nmol g-1 of freeze dry soil) of total soil microbial biomass (Gram+ bacteria, Gram- bacteria, fungi, protozoa, and cyanobacteria), of soil fungi, and of soil

bacteria (Gram+ and Gram-) of the different treatments (*B, Control (C), H, P, S,* and *W*) as well as of fungi: bacteria ratio, 52 days after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*.

	Total microbial biomass		**Fungi**		**Bacteria**		**Fungi: Bacteria**	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	1537	1170	639	528	860	631	0.7101	0.0789
C	2336	2171	1117	1127	1164	1030	0.8680	0.2510
H	2720	1633	1211	773	1453	841	0.8136	0.0790
P	2181	1744	959	805	1176	933	0.7436	0.1831
S	1764	1360	764	695	956	666	0.7270	0.2990
W	1969	1416	886	628	1041	780	0.8535	0.1108

Table S19 Phospholipid fatty acid (PLFA) compounds (nmol g-1 of freeze dry soil) of soil microbial biomass of Gram+ bacteria, Gram- bacteria, protozoa, and cyanobacteria of the different treatments (*B, Control (C), H, P, S,* and *W*), 52 days after mulch application (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*.

	Gram+		**Gram-**		**Protozoa**		**Cyanobacteria**	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	771	599	88.8	32.6	26.51	12.48	10.76	2.84
C	1056	972	108.2	60.1	33.70	23.50	21.58	21.69
H	1325	786	127.9	56.8	40.00	17.20	16.02	7.32
P	1069	886	107.3	47.2	32.07	15.42	14.15	8.26
S	849	647	106.9	31.5	30.75	18.77	12.86	11.23
W	943	734	98.6	47.2	31.36	10.81	10.38	3.06

Table S20 Seed C, N, and protein (%) content of the wheat plants of the different treatments (*B, Control (C), H, P, S,* and *W*) (N = 5). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*.

	Seed C		**Seed N**		**Seed protein**	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	45.108	0.098	2.647	0.147	15.089	0.842
C	45.109	0.053	2.566	0.232	14.629	1.320
H	45.072	0.127	2.515	0.225	14.337	1.283
P	45.114	0.180	2.503	0.257	14.266	1.463
S	44.999	0.169	2.571	0.178	14.659	1.019
W	45.107	0.150	2.610	0.298	14.875	1.699

Table S21 Dry mass of 1000 seeds (g) (N = 5), ear length (cm) (N = 75), and ear dry mass (g) (N = 75) of the wheat plants of the different treatments (*B, Control (C), H, P, S,* and *W*). The residue types were:

Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Mean values that do not share a common letter are significantly different ($p<0.05$).

	Dry mass of 1000 seeds (g)		Ear length (cm)		Ear dry mass (g)	
Treatments	**Mean**	**StDev**	**Mean**	**StDev**	**Mean**	**StDev**
B	35.490	1.871	11.215[a]	0.873	3.033	0.800
C	36.819	1.664	10.096[b]	1.143	2.762	0.773
H	34.000	3.260	10.888[a]	1.041	2.814	0.712
P	33.836	1.831	10.979[a]	0.961	2.739	0.887
S	35.940	2.700	10.731[a]	1.196	2.857	0.890
W	39.810	5.580	10.133[b]	1.134	2.728	0.662

Table S22 Significant and nearly significant differences of ear length (cm) between the different Tukey's post- hoc test for differences between treatments (*B, Control (C), H, P, S,* and *W*) in ear length (cm) ($N = 75$, $F = 14.06$, p-value < 0.001). The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values <0.05 indicate significant differences

Difference of Levels	**T-Value**	**Adjusted P-Value**	**Difference of Levels**	**T-Value**	**Adjusted P-Value**
C - B	-6.44	<0.001	S - C	3.65	0.004
S - B	-2.79	0.060	W - H	-4.34	<0.001
W - B	-6.22	<0.001	W - P	-4.87	<0.001
H - C	4.56	<0.001	W - S	-3.44	0.008
P - C	5.08	<0.001			

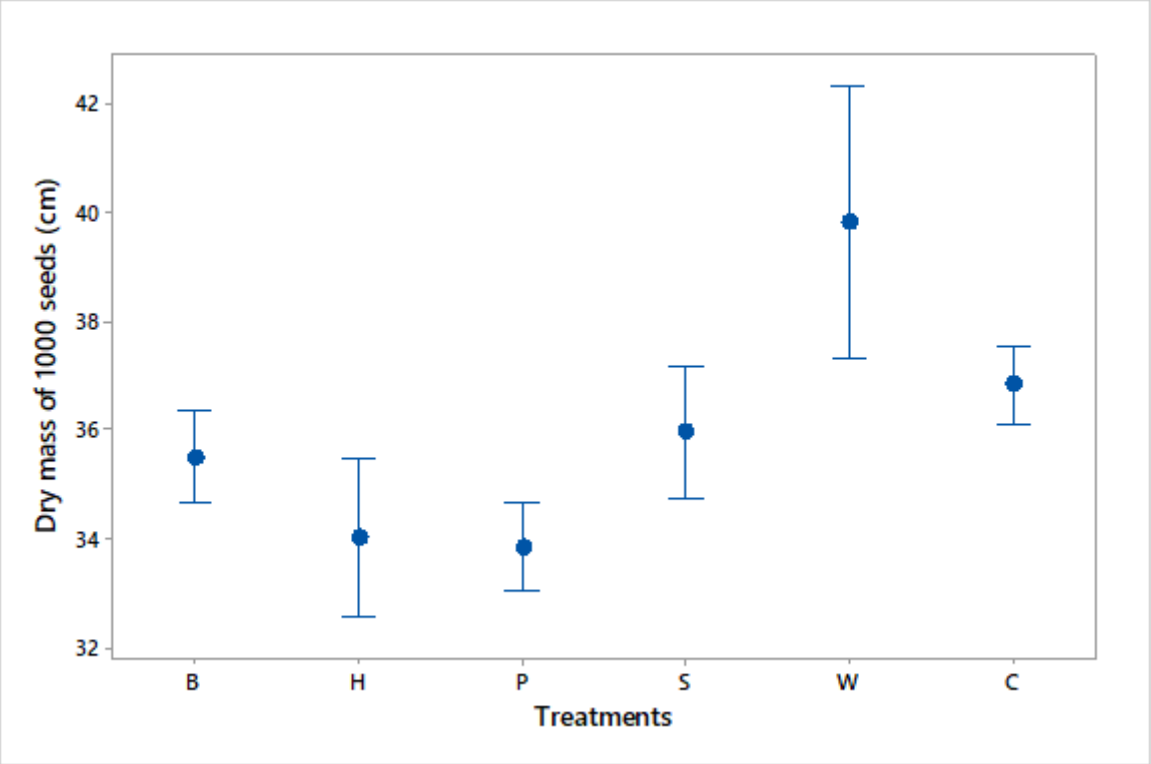

Fig. S4 Interval plot of dry mass of 1000 wheat seeds (g) of the different treatments (*B, Control (C), H, P, S,* and *W*), at harvest time. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. Bars are one standard error from the mean. Means that do not share a common letter are significantly different (p-value < 0.05)

Table S23 Spearman correlations of significant influences found between variables related with initial residue chemical composition (C, N, and C:N ratio), and with seeds and ears (seed C, seed C:N ratio, ear length, dry mass of 1000 seeds - DM of 1000 seeds, and dry mass of ears with seeds – Ear DMWS). All types were included and *Control* treatment where applicable. The residue types were: Perennial ryegrass ***(P)*** (1 plant species), Smart Grass ***(S)*** (6 species), Biomix ***(B)*** (12 species), Herbal ***(H)*** (17 species), and wood chips *(W)*. P-values are in parentheses. Correlations with p-values <0.05 are significant

Sample 1	Sample 2	Spearman correlation coefficient (ρ)	p-value
Seed C:N	Seed C	-0.598	0.002
Seed C:N	Seed N	-0.998	<0.001
Seed C:N	Seed protein	-0.998	<0.001
Seed C	Seed N	0.613	0.001
Seed C	Seed protein	0.613	0.001
Seed N	Seed protein	1.000	<0.001
Ear length	Initial residue C	-0.598	0.002
DM of 1000 seeds	Initial residue C	0.401	0.047
Ear length	Initial residue lignin	-0.458	0.021

DM of 1000 seeds	Initial residue lignin	0.424	0.035
Ear DMWS	DM of 1000 seeds	0.528	0.007
Ear DMWS	Ear length	0.441	0.027

Chapter 5

Discussion

Three experiments (exp1, exp2, and exp3) have been conducted to answer the research questions. The first and the second were in rhizotrons, and the third in field. Residue species composition was similar in the three experiments, with the addition of the wood chips treatment in the second and third experiments. However, their initial quality differed. Moreover, in exp1 residue length was of two sizes of 30 cm, and 1.5 cm, in exp2 it was 30 cm, and in exp3 it was whole plants, while wood chips in all cases were shredded to less than about 10 cm.

In exp1 the initial C:N ratio of residues was from 24.2 to 33.5. A C:N ratio ≤24 is deemed to drive to net N mineralization, although this threshold may rise up to about 35 or even 40 when soil fauna is involved in decomposition (Brust, 2019; Frouz *et al.*, 2015). In exp2 the C:N ratios of the same residue types were very low, ranged between 10.7 and 16.8, leading to direct N mineralization, while that of wood chips was 50.1 (>40) which is deemed to cause initial N immobilization (Brust, 2019). In exp3 wood chips had the same characteristics with those in exp2, while the C:N ratios of the other treatments were ranged from 12.8 to 33.5, nearly the range of the other two experiments together. In all experiments $P_{(1)}$ treatment had the lowest initial C:N ratio, and the highest initial N content, while *W* treatment had the opposite but also relatively extreme values.

Decomposition rate was significantly affected by residue chemical composition in exp1. Residue dry mass loss was significantly negatively correlated with initial residue NDF content, and in residues with high initial NDF it was significantly negatively correlated with residue initial N content, although NDF could explain only 21.12% and N content only 24.50% of mass loss variation. These findings were in line with previous research where residue mass loss was negatively affected by both residue recalcitrance (Xu *et al.,* 2017) and by residue N content, because high N availability restricted lignin decomposition due to microbial competition (Fog, 1988). Decomposition decelerates as it proceeds due to increase of recalcitrant substances (Xu *et al.*, 2017) favouring mass loss from residues of lower initial recalcitrance at later stages, regardless the C:N ratio. NDF was used as a measure of recalcitrance in all experiments because it includes both intermediately (hemicellulose and cellulose) and slowly (lignin) decomposing compounds (Hadas *et al.,* 2004). Moreover, residue size did not affect residue mass loss, which was in accordance to previous research (Ambus et al., 2001; Bremer et al., 1991). Likewise, in exp2 residue dry mass loss was higher by residues of higher initial quality, in accordance with Xu *et al.* (2017), but again low initial recalcitrance was the most influencing factor regardless the C:N ratio. This indicates clearly that recalcitrance was the most influencing factor in residue mass loss, and the influence of C:N ratio is highly dependent on recalcitrance (Vahdat *et al.*, 2011). Therefore, considering both experiments we may conclude that chemical composition of residue mixtures had

more significant effect on their decomposition than their species diversity, in agreement with Redin *et al.* (2014).

The final C:N ratio in exp1 was higher in *long-residues* than in *short* ones, and in types with higher initial recalcitrance, indicating that final residue quality was affected by both residue length and residue chemical composition. A higher C:N ratio in *long residues* than in *short* ones of the same type denotes slower decomposition rate (Spain and Hodgen, 1994). *Short residues* were decomposed faster due to increased contact of soil decomposer microbes with residue surface (Jensen, 2014). Contrary, residues of higher recalcitrance (lower quality) were decomposed slower because the dominant responsible microbes were characterized by slower nutrient cycling processes (van der Heijden *et al.*, 2008). *Long residues* resulted in higher both final C and final N content than *short residues* and considering that the residue mass loss was not significantly affected by residue size we reach the conclusion that *long residues* maintained higher fertilization capacity than *short residues* at the end of the growing season, in line with my hypothesis. In exp3 the remaining residues, in each treatment, were mixed with crop residues at harvest time, common practice in conservation tillage, producing the final residue mulch for the next crop. The C:N ratio of the initial residues was significantly lowered at harvest time (Akratos *et al.*, 2017). Therefore, the C:N ratio of the combined residues at harvest time in the different treatments was ranged from 19.6 to 26.8, producing residues of higher quality than that of the cash crop residues alone in control treatment (C:N 32.4), except that of *W* treatment which was 36. Even in *W* treatment the addition of crop residues improved final C:N ratio and kept it below 40, reducing a possible N immobilization (Brust, 2019). However, lignin content was also raised from 2.1 to 9.4 times fold (the lowest in *W* treatment) (Frouz, 2018). Increase of lignin was inversely proportional with the initial lignin and N content. The order of treatments in final C and N content was the same as in initial residues, but the differences were not significant anymore, including the *Control* treatment (wheat residues). These results confirmed the hypothesis that the remaining residues in combination with the crop residues at harvest produce residues of higher quality than those of the crop residues alone.

In exp1 there were weak correlations between final residue C and N in treatments with *long residues*, and strong relevant correlations in those with *short residues* on day 137 after mulch application (day 137), further confirming the effect of length on final residue quality. Possibly this indicates that different groups of microbial decomposers were favoured in *long* and *short residues*, but further investigation is needed to confirm this hypothesis. *Short residues* exposed larger surface to microbial decomposers, and were in more contact with soil favouring bacterial rather than fungal activity and therefore higher decomposition rates (Grosso *et al.,* 2016; van der Heijden *et al.,* 2008).

Arbuscular Mycorrhizal Fungal (AMF) colonization in barley plant roots on day 137 in exp1 was higher in treatments with higher species richness in ascending order, and control had the lowest value, although the differences were not statistically significant. However, regression analysis confirmed that

there was statistically significant positive relation between AMF root colonization and residue species richness. This was consistent with previous research (Burrows and Pfleger, 2002) and is a strong indication that residue species richness can positively affect AMF root colonization, according to my hypothesis, but no effect of residue length on AMF was found.

PLFA in exp2 showed that residues of high quality resulted on higher soil microbial biomass on day 69 than those of lower quality, probably due to higher N availability, in consistence with previous research (Tahir and Marschner, 2017), and on lower microbial biomass on day 195. Higher microbial biomass is an indication of higher rate of decomposition and therefore it shows a possibility that the decomposition was initially higher in residues of higher quality than those of lower quality at least up to day 69 and this trend was reversed sometime up to day 195, as it was hypothesised. However, this hypothesis was not fully confirmed because differences were not significant. In addition *Control* had the lowest value in soil microbial biomass on day 69, which indicates the contribution of residues to enrichment of soil microbiology, in accordance with previous observations (Shu *et al.,* 2021).

The influence of residue quality on soil microbiology was reflected on higher total soil C and N at 0-5 cm depth by residues of high quality both on days 69 and 195, although significantly different only on day 195, confirming the hypothesis that residue quality affects soil nutrient dynamics at the later stages of decomposition. This is an indication that higher rates of decomposition by high quality residues continued long after day 69 and before day 195. Fungal biomass was higher than bacterial on day 195 than on day 69 due to gradual increase of residue recalcitrance (Xu *et al.*, 2017; Liang *et al.*, 2017). However, increased C and N mineralization by high quality residues was not reflected in soil NH_4^+ content at 50-55 cm depth, which was not significantly different between treatments on both day 69 and 195, while soil NO_3^- were not detected at all. This shows an ability of mulch to provide N avoiding at the same time losses by leaching (Ambus *et al.*, 2001).

In exp3, which was a field experiment, on day 52 higher soil microbial biomass was observed in $H_{(17)}$ treatment with relatively low quality residues of high initial C content, high recalcitrance, and low N, indicating a shift in decomposer microbes from bacteria to fungi (Santonja *et al.*, 2018), probably due to much higher rates of decomposition than the other treatments that day. $H_{(17)}$ treatment was followed by *Control*, $P_{(1)}$, W, $S_{(6)}$, and $B_{(12)}$ treatments, which enhances the possibility of soil microbial biomass to be affected by residue chemical composition rather than by residue species richness. Possibly, a C:N ratio of about 33, as that of the $H_{(17)}$ treatment, was ideal for the development of soil decomposer microbial community provided both adequate quantities of labile C and N, while in other treatments either C (high quality residues) or N (low quality residues) were the limiting factors. There results show that, considering the reverse trend in exp2, it is possible in field exp3 a reversion of the trend between high- and low-quality residues on soil microbial biomass was in the middle of the process on day 52. If this was the case, the reversion of the trend was observed earlier in the field exp3

than in the rhizotron exp2. This can be attributed to much higher and diverse soil microbiological loading in field conditions (Miglécz *et al.*, 2015). Indeed, low-quality residues also demonstrated the highest soil fungi: bacteria ratio (apart from *Control*), indicating that readily available C rather than N was the limiting factor on decomposition at that time (Nguyen and Marschner, 2017). It is well known that labile C are mainly released by living roots and decomposed material (Kuzyakov and Blagodatskaya, 2015). Moreover, total soil microbial biomass was significantly negatively correlated with total soil C, on day 52, and soil C:N ratio was significantly correlated both negatively with initial residue N and positively with initial residue C:N, and ADF content, which indicates that possibly soil microbial biomass on day 52 was favoured by residues of higher quality. However, the hypothesis that soil microbial biomass was affected by residue quality was not fully confirmed because the differences were not significant, as in exp2.

Residues with the highest N content ($P_{(1)}$ treatment) in exp3 resulted in the highest total soil N and the lowest total soil C on day 52, in agreement with exp2, although differences between treatments were not significant. Soil NH_4^+ in exp3 at 30-45 cm depth on day 25 was higher than on day 90 as was expected, because normally they decline as the decomposition proceeds (Castro and Whalen, 2016). The opposite was true for NO_3^- which is normally produced by NH_4^+ through nitrification over time (Justes *et al.*, 2012). $P_{(1)}$ treatment which had the highest residue quality (low C:N ratio, high N, low recalcitrance) demonstrated the highest values in both NH_4^+ and NO_3^- considering both days 25 and 90 together, in accordance with previous research (White *et al.*, 2017), statistically significant in the case of NO_3^-, confirming the hypothesis that residues of higher quality result in higher N mineralization. Moreover, soil NO_3^- on day 25 was significantly correlated both positively with initial residue N, and negatively with initial residue C:N, C, and NDF content. Soil NO_3^- were higher in descending order for residues with high N content, followed by residues with low N, high recalcitrance, but low lignin content, followed by *Control* and *W* treatment which had the lowest quality (Truong and Marschner, 2018). This pattern was the same on both days 25 and 90. However, apart from $P_{(1)}$ treatment, *W* treatment and *Control* displayed high soil NH_4^+ content on both days 25 and 90, while $H_{(17)}$, $B_{(12)}$, and $S_{(6)}$ treatments had lower values. This implies either an early acceleration of decomposition due to higher and more diverse microbiological loading in field conditions, in agreement with the observations on the fugal: bacterial ratio, or a later nitrification process in more recalcitrant residues (Table S27 of exp2, and Tables S14, S15, and S17 of exp3). The later assumption is consistent with findings in incubation exp2, although in both cases the differences between treatments were not significant.

In exp1, soil K and Zn were the only nutrients (between K, P, Mg, Fe, Mn, Zn, and Cu) that were significantly affected either by residue quality or by residue size, respectively, according to the hypothesis. Soil K was higher in all treatments with residues than in *Control* in samples received from both 0-5 cm and 20-25 cm depths on both days 70 and 137. Opposite to other macronutrients (P, and

Mg), K content was higher at 0-5 cm than at 20-25 cm depth. The same was true for micronutrients Fe, Mn, Zn, and Cu (Fageria et al., 2002). At 0-5 cm depth on day 137, soil K in $S_{(6)}$ residue type was significantly higher than in $B_{(12)}$ and $P_{(1)}$ types. $S_{(6)}$ type had the highest initial C:N ratio, high recalcitrance, and the lowest N content (low quality residues), while $B_{(12)}$ and $P_{(1)}$ types had either the lowest C:N ratio or the lowest recalcitrance. Spearman correlation confirmed that there was a statistically significant positive correlation between soil K and initial residue C:N ratio, in line with the hypothesis that nutrient availability is affected by residue quality. High quality residues release K in soil at early stages of decomposition, while low quality residues at later stages (Campbell *et al.*, 1993). Plant residues are an important source of enrichment of soil with K (Singh and Khind, 1992). K is readily released to soil from plant residues because it exists into plant tissues as K^+ ion, and it is easily subjected to leaching (Xie *et al.*, 2020). Soil K content was decelerated over time, and it was higher on day 70 than on day 137, in agreement with Reichert *et al.* (2015). Soil Zn content was higher at *short residues* than at *long* ones on day 137 at 0-5 cm depth, confirming the hypothesis that nutrient availability is affected by residue size. Zn deficiency is often in cereal cultivars with adverse effect on crop yield (Genc *et al.,* 2002). In such conditions Zn can be replenished by phytosiderophore exudates from barley plants (Li *et al.*, 2014), and possibly this was the case in *short residues*. Contrary, if *short residues* did not cause Zn deficiency, it is possible *long residues* which have a slower decomposition rate would produce higher amount of soil Zn at later stage.

In exp2 soil nutrients other than N (P, K, Mg, Mn, Fe, Zn, and Cu) were measured on day 69. Soil macronutrient (K, Mg, P) contents were higher at 20-25 cm depth, and micronutrients (Fe, Cu, Mn) except Zn were higher at 0-5 cm depth, on day 69. Wheat cultivation resulted in decrease of soil K, and Mg, and increase of soil Fe, Cu, and Zn, in comparison to the bulk soil prior to the experiment. All treatments with residues had higher values than *Control* of all soil nutrients, except Cu, and Zn. This implies a positive contribution of plant residues in soil nutrient balance in all nutrients. Soil K was the only nutrient that was significantly affected by residue quality, in consistence with the hypothesis, likewise in exp1. All treatments had significantly higher values of soil K than *Control* at 0-5 cm depth. At 20-25 cm depth $H_{(17)}$ treatment, characterized by high C:N ratio, low N, and high recalcitrance, had the highest value, significantly different than *Control*. This finding was in consistence with that in exp1 and it shows the significance not only of the high-quality residues but also of those of lower quality, applied as mulch, in enrichment of soil with essential nutrients at later stages of decomposition. However, in contrast to exp1, in exp2 regression analysis did not find any significant influence of initial residue C:N ratio on soil K.

Barley yield was neither affected by residue quality nor by residue size in exp1. The same conclusion for the effect of residue quality on yield was drawn by exp2, and these results were in consistence with the hypotheses. In previous experiments controversial results on crop yield were reported. Peterson *et al.* (1998) observed a small reduction on wheat yield in mulch application in comparison to

incorporation of residues by ploughing. Reichert *et al.* (2015) found no significant differences in crop yield between treatments of diverse chopped material, while Awopegba *et al.* (2017) observed significant differences in crop yield between treatments of diverse residues.

Long residues in exp1 demonstrated higher stem elongation rates at barley plant growing stage GS30 to GS39 (rapid stem elongation stage) (Tottman et al., 1986) than *short residues*. Regression analysis showed a statistically significant positive relation between stem elongation rate at the growing stage GS30 to GS39 and seed protein content, which confirmed the hypothesis that crop quality can be affected by residue size. *Long residues* are deemed to be decomposed in slower rate than *short* ones, resulting in lower available N for soil microbes (Giacomini *et al.*, 2007). This is true for the initial short-term decomposition stage and this trend is reversed at later stage (Bremer et al., 1991). Gradually, soil fauna breaks *long residues* into shorter particles, breaking recalcitrant compounds, enhancing their contact with microbial decomposers, and raising nutrient release (Angers and Recous, 1997). Possibly, *long residues* released higher amount of N at GS30 to GS39 than *short residues*, evidenced by higher stem elongation rate. High available N at GS37 is crucial for a high seed protein content because it is stored in barley plants and is transferred to ear and seed development at GS59 to GS87 (Boyle, 2017). The effect of residue size on seed protein content should be further investigated as it is of great interest for both farmers and food processors. Moreover, a significant negative correlation was found between seed protein content and length of ears, which shows that residue size may also affect morphological traits of crop plants.

In exp2 differences between treatments in main stem elongation rate were not significant at any timepoint. However, Spearman correlation revealed statistically significant negative correlation between initial residue C:N ratio and seed C content, which was statistically significantly positively correlated with seed protein content, and this was statistically significantly negatively correlated with crop yield. Therefore, although the only significant differences between treatments were in seed C content, considering these correlations we may reach the conclusion that residues of higher quality (low C:N ratio) resulted in higher seed C, higher seed protein, and lower yield than residues of lower quality.

In exp3 residues chemical composition significantly affected wheat crop morphology. Both residue C and lignin content were significantly negatively correlated with wheat ear length and significantly positively correlated with seed weight. In contrast, there was no evidence of an effect of residue chemical composition on seed quality. Seed protein was significantly positively correlated with both seed N and seed C, but seed N and C were not significantly correlated with initial residue C and N. However, considering that in both exp2 and exp3 seed C was positively correlated with seed protein someone could hypothesise that as in the rhizotron so in the field experiment the high-quality residues (low C:N ratio) could result in higher seed C and seed protein content than low quality residues, which

agrees with previous research (Awopegba *et al.,* 2017), but this hypothesis needs to be further investigated. PCA analysis in both exp2 and exp3, considering variables related with crop quality (including crop morphology), where distinctive groups were formed, confirmed that there was a significant influence of residue quality in crop quality.

The results from all three experiments show that *long residues* maintain higher fertilization capacity than *short* ones at the end of the growing season without deleterious effect on crop yield. Residue quality may affect crop quality, which is important for both food producers and food processors. In field conditions, the resulting residue mulch in combination with cash crop residues at the end of the growing season produces residues of higher quality than those of the cash crop alone. However, further investigation is needed in both incubation and field experiments with continuous application of diverse whole-plant residue mulch to evaluate optimal amounts, and appropriate timing to gain maximum benefits.

Research limitations

If I had to reschedule my research and if it was technically feasible, I would consider the following issues:

1) The plant residues that were used in the experiments were derived from plants cultivated elsewhere than in the experimental plots or in rhizotrons. Therefore, the effect of root decomposition of plant residues on the successive cash crop was missing. The ideal, at least in field experiment, would be to cultivate the cover crop plants (to be used as residues) in the experimental plots, then sow the seeds of the cash crop just before termination of cover crops, and finally cut cover crop plants and let them decompose as mulch. The cash crop plants would emerge among plant residues. Alternatively, cash crop seedlings could be planted among cover crop residues. In incubation experiments this could be approached by cultivating cover crop plants in large incubation plots of at least 0.5 m depth and appropriate surface dimensions. The overground cover crop plant biomass would be removed to be used as mulch in rhizotrons soon after cover crop termination, while the rest of the soil would be homogenized to be used as soil in rhizotrons.

2) Plant residues labelled with ^{13}C and ^{15}N stable isotopes could be used to estimate C and N mineralization from the different treatments. However, it was very costly and time consuming to be trained and apply this method, and much more to ensure technical support.

3) I would possibly exclude incubation experiments and I would concentrate in field conditions with successive crops at the same experimental plots, in order to obtain long-term data from continuous applications of whole-plant residue mulch.

There were also a few technical issues which acted as limitations in my experimental work:

1) I was not able to gather more data from Mehlich 3 analysis (Mehlich, 1984) on soil nutrients from the second and the third experiments, although sample analysis was in the middle of the process, due to pandemic restrictions in laboratory. Although it was not essential for the completion of the experiments, this data could provide additional interesting information.

2) The first attempt to grow barley plants on pots for the second experiment was failed due to poor seed germination capacity, but mulch had already been applied. Therefore, I had to repeat the process with new seeds and this fact delayed barley growth in relation with mulch application timepoint. This could be avoided if a seed germination test were conducted before sowing the seeds.

3) In incubation experiments rhizotrons had to move from the outdoor into greenhouse in September due to weather conditions in order plants reach maturity. This put the plants in abnormally high temperatures during maturity, therefore plant nutrient uptake and soil microbial activity may be deflected from those under natural conditions. It is possible, earlier inauguration of these two experiments could provide different results in some cases more approximated to natural conditions.

4) Signals in gas chromatography considered in PLFA analysis were those according to the standards provided by the manufacturer. There was not able to add more signals in analysis due to the lack of more standards. Moreover, pandemic restrictions in laboratory limited technical support. Therefore, PLFA data was not fully exploited, but this is a very common situation as shown by the relevant literature.

5) The field plot in experiment 3 was sown in late March with wheat, while mulch was available and applied in late May. It was supposed that wheat plants would tolerate the load of mulch biomass and would grow up through it. Although this was the case for the majority of plants a few of them were damaged and this spoiled the uniformity of plant density in the field plots, making it risky to estimate differences in yield. This was the reason why yield performance was not included in results. This could be avoided if mulch would be applied with sowing, about two weeks before wheat emergence, but mulch was not available that period.

6) One of the reasons to use rhizotrons was the ability to observe differences between treatments in root growth. However, in both rhizotron experiments barley roots were proved to be too tiny or too thin to provide distinctive differences with the use of an appropriate software (as image j). It would be more prudent another crop species with more thick roots to be used for this purpose. Nevertheless, rhizotrons were suitable to collect soil samples from different depths of the soil profile at different time-periods without destructing experimental units.

Conclusions

Rhizotron experiments exp1 and exp2 showed that residue mass loss was significantly affected by residue chemical composition but not by residue size. Residue mass loss was significantly negatively

correlated with initial residue NDF and N content. In exp1, *long* residues were decomposed slower than *short* ones, resulting in higher C:N ratio, but also higher C and N content, at the end of the crop growing season, 137 days after mulch application (day 137), implying higher fertilization capacity for the successive crop, considering the residue mass loss was not significantly different between *long* and *short residues*.

There were no significant differences in total soil N or in soil inorganic N between treatments in exp1. In exp2, residues of higher quality resulted in significantly higher total soil N than in *Control* at 0-5 cm depth on day 195, while there were no significant differences between treatments on soil inorganic N content. In the field exp3, there were only indications that residues of higher quality could result in higher total soil N than residues of lower quality at 0-5 cm depth, and the opposite was true for total soil C on day 52, while residues of higher quality resulted in significantly higher NO_3^- content than *Control* at 30-45 cm depth considering both days 25 and 90.

In exp1 soil K was the only nutrient, apart from N, that was significantly affected be residue quality, and soil Zn by residue size. Soil K was higher in all treatments with residues than in *Control*, and significantly higher in residues of higher initial C:N ratio, at 0-5 cm depth on day 137. A statistically significant positive correlation between soil K and initial residue C:N ratio was observed. Soil Zn was significantly higher in *short residues* than in *long* ones at 0-5 cm depth on day 137. In exp2 soil K was again the only nutrient, apart from N, that was significantly affected by the different treatments. *Control* had significantly lower value than any treatment with residues at 0-5 cm depth, and significantly lower value than in the treatment with the highest C:N ratio, but not from the wood chips with C:N ratio >40, at 20-25 cm depth, on day 69. These results show that all treatments with residues, even the wood chips, contributed to enrichment of soil K, but, in contrast to exp1, in exp2 no statistically significant correlation between soil K and initial residue C:N ratio was found.

There was a statistically significant positive correlation in exp1 between AMF root colonization and residue species richness at the end of the growing season, although differences between treatments in AMF root colonization were not significant. In exp2 clearly residues of higher quality demonstrated higher soil microbial biomass than residues of lower quality in all microbial groups at 0-5 cm depth on day 69, while the opposite was true on day 195, although differences between treatments were not significant. These microbial patterns were indications of a reverse trend in decomposition rate between residues of higher and of lower quality. Considering that residue mass loss was lower in lower quality residues, this reversion in decomposition rate implies that residues of lower quality maintained higher fertilization capacity than those of higher quality on day 195. In field exp3 residues with C:N ratio 33 had higher soil microbial biomass on day 52 than residues with higher or lower C:N ratio and *Control*, which shows it is possible that residue decomposition was at the middle of a reverse trend between high- and low-quality residues, but differences were not significant. There was also an

indication that soil microbial biomass on day 52 was favoured by residues of higher quality. The remaining residues in combination with the crop residues after harvest resulted in residues of significantly lower or not significantly higher C:N ratio and not significantly different lignin content, therefore in residues of higher or at least equal quality, than those of the crop residues alone.

In both exp1 and exp2 crop yield was not significantly affected either by residue size or by residue quality. In exp1 *long residues* resulted in higher stem elongation rate than *short residues* at the stage of the rapid stem elongation, where N availability is determined for a high seed protein content, which is of great importance for both food farmers and food processors. Mean values of seed protein were higher in *long residues* than in *short* ones, but differences were not significant. Residues of higher quality in exp2 resulted in significantly higher seed C content which was significantly positively correlated with seed protein. In exp3 wheat crop ear length was significantly shorter in Control and in wood chips than in any other treatment. Ear length was significantly negatively correlated with both residue C and residue lignin content, and significantly positively correlated with the wheat seed weight, but residue quality characteristics (C:N, C, N, NDF) were not significantly correlated with seed C, N, or protein content. Therefore, considering all the three experiments, we draw the conclusion that both residue size and residue quality may affect more readily crop quality (crop morphology including) rather than crop yield.

Generally, the three experiments showed that long residue mulches were more advantageous than short residues in maintaining soil fertility. Residues of both high and low quality were proved valuable at later stages of decomposition. Residue mass loss from decomposition of diverse mulches at the end of the growing season was higher in residues of lower recalcitrance rather than those of higher quality and was not affected by residue size. Long residues maintained higher fertilization capacity at harvest, and residues of lower quality at a timepoint long after harvest. In field conditions the remaining residues were capable to improve crop residues at harvest. Soil nutrient dynamics were affected by both residue quality and residue size. There were indications that during decomposition soil microbial biomass was initially higher in residues of higher quality than those of lower quality, but the trend was reversed at later stages. AMF root colonization was significantly positively correlated with residue species richness. Both residue size and residue quality affected crop quality rather than crop yield. More research is needed with continuous applications of whole-plant diverse mulches to further investigate the role of the significant functional traits of residues related with their decomposition, and the appropriate amounts and number of applications in combination with different environmental conditions to optimize benefits for a sustainable agriculture.

Summary

The main interest of my research was to investigate the potential of diverse *long-size* plant residues applied as mulch to improve soil fertility and to be used as a fertilizer to provide satisfactory crop yield. Soil fertility is a combination of physical, chemical, and biological soil properties.

Initially, I wanted to examine whether the shredded, *short residues*, which have been studied more extensively than the *long residues* mainly in incubation experiments, have an advantage in improving soil fertility or have been overestimated in relation to *long residues* (>30 cm length or whole plants), especially when it comes to long-lasting effect. At the same time I wanted to see if the diversity of residues in terms of the species richness (number of species) or of the species composition, and the residue quality (residue chemical composition) also plays an important role in this. For this reason I focused mainly on the study of later stages of residue decomposition as well as on the final characteristics of residues (C:N ratio, C, N, recalcitrance, mass loss) of the various treatments at the end of the growing season in order to evaluate, where possible, the remaining fertilization capacity of the residues that could affect a successive crop. Finally, I wanted to see if there were similar effects both in incubation experiments and in field conditions.

To answer these questions I conducted 2 incubation (rhizotron) experiments and a field experiment. In the first rhizotron experiment I focused on the effect of residue size and examined the hypothesis that either residue size or residue quality or residue diversity will affect final residue quality and consequently residue fertilization capacity, soil nutrient availability, Arbuscular Mycorrhizal Fungi (AMF) root colonization of barley plants, and probably barley seed quality but not barley plant yield. The results revealed that residue mass loss was affected by residue chemical composition and not by residue size. In addition *long residues* had higher final C and N content and hence higher fertilization capacity than the *short residues* at the end of the growing season. Soil K was significantly affected by residue quality and Zn by residue size, while there were indications that residue size could also affect seed quality, and residue species richness could positively affect AMF root colonization and probably soil microbial population in general. Therefore, *long residues* seemed to provide more benefits in total than *short* ones. They seem to have the capacity to provide more nutrients to the successive crop, while at the same time they can improve soil physical properties more effectively than the short residues because they can cover the soil surface for longer period due to lower decomposition rate (Angers and Recous, 1997). Therefore, they can more effectively protect the soil surface from the solar radiation and from the rain drops, protect from soil erosion, increase water infiltration, soil porosity, soil aggregation stability, soil aeration, and mediate soil temperature oscillation (Su *et al.*, 2014; Mulumba & Lal, 2008; Sharma *et al.*, 2011; Jordán *et al.*, 2010; Blanco-Canqui and Lal, 2009).

Considering these results I designed the second rhizotron experiment using only *long residues* but also a treatment of wood chips. Wood chips were characterized by extreme values of chemical traits such as C:N ratio and recalcitrance in comparison to the other treatments that could highlight significant

differences between treatments. In addition they are often used for mulching, especially in organic farming or agroforestry, where they exist in abundance as a result of tree pruning. The main purpose of the second experiment was to examine the effect of the diversity and of the quality of *long residues* on the rate of decomposition, on the availability of soil nutrients, and on the soil microbial populations at later stages of decomposition, on the crop quality and the crop yield, as well as to evaluate the residue fertilization capacity at the end of the growing season at a timepoint long after harvest. Based on the relevant literature I assumed that *long residues* of higher quality are initially decomposed faster than *long residues* of lower quality, but the rate of decomposition is reversed at later stages resulting in significant differences in soil microbial biomass and in nutrient dynamics but not in crop yield. In addition, I hypothesised that residues of lower quality demonstrate higher fertilization capacity than those of higher quality at the end of the growing season at a timepoint long after harvest. The results provided an indication that residues of higher quality led to higher microbial biomass initially, but this trend was reversed at later stage, because differences between treatments were not significant although the patterns were clear. Moreover, residues of higher quality had higher dry mass loss, implying that residues of lower quality could possibly maintain higher fertilization capacity than residues of higher quality at the end of the growing season at a timepoint long after harvest. Again, crop yield was not affected by residue diversity or residue quality, but the crop quality was affected by residue quality.

In the third experiment that took place in the field I used the same treatments as in the second one while at the same time I had the opportunity to use whole-plant residues cut once on the soil surface since there was no space limitation for mulching application as in the rhizotron experiments. Field experiments are important as they involve unexpected and often uncontrollable factors such as environmental variability, changing climate conditions and inhomogeneity in mulching application on soil surface. The results showed that there were indications that the remaining residues at harvest time in combination with crop residues can provide residue mulch of higher or at least of equal quality than that of the crop residues alone. The soil microbial biomass was not significantly different between treatments. Only the ear length of the wheat crop was significantly different between treatments. It was shorter in Control and in wood chips than in any other treatment. Ear length was significantly negatively correlated with both residue C and residue lignin content, and significantly positively correlated with the wheat seed weight, but residue quality was not significantly correlated with seed C, N, or protein content.

Considering all three experiments, I concluded that, long residues may provide advantages in maintenance of soil fertility in comparison to the short residues, without a deleterious effect on crop yield. Residues of higher quality are initially decomposed faster but those of lower quality are evenly valuable for they provide benefits at later stages of decomposition. In field the remaining residues combined with the crop residues possibly may improve the final residue mulch quality. AMF root

colonization possibly may be affected by residue species richness. Soil microbial biomass may be affected by residue quality, but differences between treatments may not be significant. Crop quality may be significantly affected by residue quality. However, further research is needed with continuous mulch applications with diverse *long residues* in order to determine the optimal number and amount of residue applications in a growing season, and the desirable residue characteristics at different environmental conditions to increase soil fertility.

List of publications in scientific journals with peer review

Gaitanis, D., Papadopoulou, M. (2017). Environmental Impacts Assessment of Conventional vs Organic Viticulture Using Life Cycle Assessment. *Hydrotechnika – journal of the Hellenic Hydrotechnical Association: Volume 26 (p.53-66).* Thessaloniki.

Research skills training

I have attended the following Reading Researcher Development Programme (RRDP) and other courses in UoR:

- RRDP - How to Write a Paper
- RRDP - Ensuring confirmation of registration
- RRDP - Statistical modelling and graphics using R
- RRDP - Preparing Posters - theory (part 1) & practical (part 2)
- RRDP - Managing data & research material
- RRDP - Writing up your data analysis
- RRDP – Sourcing information for a literature review – information retrieval
- RRDP – Basic statistics refresher
- Preparing to Teach (P2T)
- Academic Writing Essentials and Practice
- Presentation Skills
- Electron Microscopy
- EMA Early adopters, training practice course: Blackboard
- EMA Early adopters, training practice course: Turnitin

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