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Belowground interactive effects of elevated CO_2, plant diversity and earthworms in model grasslands

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ABSTRACT

The potential interactive effects of future atmospheric CO_2 concentrations and plant diversity loss on the functioning of belowground systems are still poorly understood. Using a microcosm greenhouse approach with assembled grassland plant communities of different diversity (1, 4 and 8 species), we explored the interactive effects between plant species richness and elevated CO_2 (ambient and + 200 p.p.m.v. CO_2) on earthworms and microbial biomass. We hypothesised that the beneficial effect of increasing plant species richness on earthworm performance and microbial biomass will be modified by elevated CO_2 through impacts on belowground organic matter inputs, soil water availability and nitrogen availability. We found higher earthworm biomass in eight species mixtures under elevated CO_2, and higher microbial biomass under elevated CO_2 in four and eight species mixtures if earthworms were present. The results suggest that plant driven changes in belowground organic matter inputs, soil water availability and nitrogen availability explain the interactive effects of CO_2 and plant diversity on the belowground compartment. The interacting mechanisms by which elevated CO_2 modified the impact of plant diversity on earthworms and microorganisms are discussed.

Keywords: Climate change; Species richness; Above-belowground interactions; Water availability; Soil N; *Lumbricus terrestris*

Introduction

Understanding of the relationship between biodiversity, climate change and the ecosystem services upon which we are heavily reliant emerged as a critical issue in the face of increasing human-induced environmental change (Millennium Ecosystem Assessment 2005). Whilst much attention has been devoted to separately studying the impacts of climate change and biodiversity loss, considerably less is known about their interactive effects on ecosystems and the services they provide (Balvanera, Pfisterer, Buchmann, He, Nakashizuka et al. 2006). Studies which include the effects of climate change factors on the functioning of the belowground compartment are even fewer and by and large highlight the considerable lack of knowledge in this area (Chung, Zak, Reich & Ellsworth 2007, Kardol, Cregger, Campany & Classen. 2010). Since the biological functioning and the species composition of the belowground compartment is dependent on organic matter inputs from photosynthesising plants, the understanding of the links between producer and decomposer subsystems is essential for predicting of ecosystem-level responses to global change (Wardle 2002).

Grasslands constitute a widespread type of ecosystem covering ~ 27% of terrestrial surface (Henwood 1998), highlighting their importance as model systems used for investigating the consequences of biodiversity loss (Roscher, Temperton, Scherer-Lorenzen, Schmitz, Schumacher et al. 2005, Tilman, Reich & Knops 2006) or elevated CO_2 (Niklaus, Alphei, Kampichler, Kandeler, Körner et al. 2007, Reich 2009). Reich, Knops, Tilman, Craine, Ellsworth et al. (2001) showed that plant diversity interacts with elevated CO_2 and nitrogen availability to modify ecosystem functioning. Furthermore, elevated atmospheric CO_2 concentrations usually reduce stomatal density and conductance (Woodward & Bazzaz 1988; Tricker, Trewin, Kull, Clarkson, Eensalu et al. 2005) which may result in increased soil moisture by reducing evapotranspiratory loss of water (Morgan et al. 2001; Nelson, Morgan, Lecain, Mosier, Milchunas et al. 2004) under elevated levels of CO_2. Although both CO_2 and

plant diversity loss have been shown to impact the decomposer functioning through changes in the quality and quantity of organic matter inputs and impacts on soil moisture and nutrient availability, we have a limited understanding of their combined effects on the decomposer functioning (Niklaus et al. 2007) despite their important role in biogeochemical cycling, plant performance and soil C storage (Bardgett 2005).

Both earthworms and microorganisms are key decomposer groups sensitive to changes in the aboveground inputs, as well as soil water and nutrient regimes. Currently we have only a limited mechanistic understanding of the interactive effects of elevated CO_2 and plant diversity loss on their performance. The few studies which report effects of elevated CO_2 on earthworms predominantly measure their response in terms of activity, indicating increased activity with increasing levels of CO_2 (Yeates, Tate & Newton 1997, Arnone & Zaller 1997; Zaller & Arnone 1999c). However, an observation of community biomass and composition by Zaller & Arnone (1999c) found no effects of elevated CO_2. Available data also show that increasing plant species diversity (Zaller & Arnone 1999b; Speh, Josh, Schmid, Alphei & Körner 2000; Niklaus et al. 2007) has a positive effect on earthworm biomass and activity, but depends on species-specific relationships with earthworms (Zaller & Arnone 1999c; Milcu, Partsch, Langel & Scheu 2006). Milcu et al. (2006) suggested that changes in the quality rather than quantity of plant inputs associated with changes in species diversity affect earthworm performance. The consequences of elevated CO_2 on microorganisms are still unclear due to insufficient data to predict how microbial biomass and functioning change as atmospheric CO_2 concentration continues to rise (Zak et al 2000, Bardgett, Freeman & Ostle YEAR). The effect of diversity loss on soil microorganisms is somewhat clearer with recent publications pointing out the positive relationship between plant diversity and the functioning and biomass of microbial communities (Eisenhauer et al 2009).

The scope for interacting effects of diversity loss and elevated CO_2 on soil organisms is clearly large. Climatic change is likely to both directly and indirectly (via plant responses) affect soil biota, with associated consequences for ecosystem functioning. In this study we explored the interaction between plant species richness, elevated CO_2 and the presence of earthworms and its effects on the belowground compartment of model grassland, using a microcosm greenhouse experiment. Our overarching hypothesis is that the frequently observed beneficial effects of increasing the richness of plant species on earthworm performance and microbial biomass will be altered by elevated CO_2, through changes in belowground organic matter inputs and altered soil water and nitrogen availability.

Materials and methods

Experimental set up

Experimental grassland communities of varied plant species richness (1, 4 and 8 species) were established in cylindrical microcosms made of PVC tubes, 10.3 cm in diameter and 25 cm in height. The microcosms were sealed at the bottom with 0.5mm^2 mesh, and a layer of sand (0.25kg) was added to the bottom of the microcosms to ensure efficient drainage. The sand was topped by 1.6 kg of sieved soil (1cm diameter sieve) taken from the A horizon of a mesotrophic grassland (MG6 – Lolium perene - Cynosurus cristatus; Rodwell 1998) in Silwood Park, Ascot, UK (N 51° 24.3 W 00° 38.5). Prior to use, the soil (bagshot sands, pH 5.35) was homogenized and defaunated by freezing at -22^{o}C for two weeks (Huhta, Wright & Coleman 1989). Subsequently, the soil was watered intermittently over a 12 day period with deionised water to remove excess nutrients released by the perturbation.

The microcosms were planted out with eight seedlings (~ 5 cm height) pre-germinated in John Innes compost from a species pool of 37 grassland species (Appendix 1). The microcosms were assigned to eight air-conditioned chambers (100cm x 100cm x 53.5

cm) located in a greenhouse. An elevated atmospheric CO_2 treatment of 600 ppm simulating the average prediction for 2100 (IPCC 2007) was applied to four of the chambers, with the other four receiving air with ambient concentration of ~400 ppm.

The earthworm treatment (EW) was established by adding one *Lumbricus terrestris* L. (4.9774 ± 0.067g FW) to one set of the microcosms in each chamber. *L. terrestris* was chosen as a typical representative colonising the grassland type (MG6) from which the soil was extracted. As *L. terrestris* is an anecic litter feeding species, 3g DW of *Dactylis glomerata* litter (cut into 3cm pieces) was placed onto the soil surface in each microcosm. No additional litter entered the microcosms as any plant litter originating from the aboveground biomass was removed weekly. Each growth chamber thus contained 36 microcosms: two replicates of each species mixture, one containing a *L. terrestris* individual (EW) and one control (NW). The resulting total of 288 microcosms (3 plant diversity levels × 6 replicates/diversity level × 2 earthworm treatments × 2 CO_2 levels × 4 replicates per CO_2 level) were fitted with 15cm high cylindrical transparent plastic guards at the top of each microcosm to contain the earthworms in the microcosms. The greenhouse was lit naturally, but also received additional light for 8 hours a day via 400 Watt overhead 'POOT' lamps. Identical environmental conditions were maintained in all chambers, apart from the 4 chambers where atmospheric CO_2 was maintained at 600ppm for 8 hours during the photosynthetically active part of the day. Air humidity, air temperature and CO_2 levels of each chamber were monitored continuously. The microcosms were watered every 3 days, initially with 30ml of deionised water, increasing to 60ml, 80ml and then 120ml over the course of the experiment to account for increasing water use by growing plants. The positions of the microcosms within the growth chambers were randomised every 14 days.

Plant diversity experimental design

A pool of 37 South-East England mesotrophic acid grassland plant species belonging to three functional groups (grasses, herbs and legumes) was used to produce a total of 18 different species mixtures (see Appendix A) using independent random draws with replacement (i.e. species mixtures were created by randomly selecting species from the species pool with all species being available for to be drawn for any species mixture). The established gradient of species diversity (1, 4 and 8) had 6 replicates per diversity level. Any sampling effects were reduced by using species pool much larger than the maximum diversity level (Aarssen 1997).

Sampling and analytical procedures

The experiment has been harvested after four months of CO_2 exposure when the majority of plants showed signs of senescence. Immediately prior to destructive sampling, the soil moisture level of each microcosm was measured (ThetaProbe Soil Moisture Sensor - ML2x, Delta-T Products). Soil C/N content was analysed using a CNS elemental analyser (Thermo Scientific FlashEA 1112 series). Earthworms were hand collected during the final harvest, washed, dried for 1 min on filter paper and weighed. A sub-sample of soil was sieved to remove any roots (2mm dia.) and stored at 4^oC. After 2 weeks the soil was used to assess the microbial biomass using the substrate induced respiration (SIR) method (Anderson and Domsch, 1978) using electrolytic O_2 microcompensation apparatus (Scheu 1992). The microbial respiratory response to the addition of glucose was measured at 30-minute intervals for 24h at 22^oC. Sufficient amount of glucose in solution was added to the substrate in order to saturate the catabolic activity of microorganisms (4 mg glucose per 1g of soil dry weight). The maximum initial respiratory response (MIRR [µg O2*h-1*g soil dw-1]) was calculated as the average of the lowest three readings within the first 11 h and microbial biomass (C_{mic} [µg C_{mic}*g soil dw-1]) was calculated as MIRR*38*0.7 (Anderson & Domsch 1978).

Statistical analysis

Mixed effects models, as implemented in the R Statistical package (lme function, R version 2.10.1), was used to analyse the fixed effects of CO_2 (CO_2), earthworm presence (EW), plant species richness (SR) and their interactions on earthworm biomass, microbial biomass, soil moisture and soil N and C/N ratio. The random-effects of the maximal model, fit by maximum likelihood, had a microcosm within chamber random structure to account for the nested design [model <-lme (y ~ CO_2*EW*SR, random = ~1 | chamber / microcosmID), where y indicates the response variable, chamber represents the eight growth chambers at which the CO_2 treatment has been applied and microcosmID represents the individual microcosms]. Minimum adequate models were achieved by sequentially excluding non-significant terms (starting with highest-order interactions) and comparing each model with its predecessor using Akaike information criterion (Crawley 2007). Tukey's HSD posthoc test was used to perform pairwise comparison of treatments with more than two levels. Several continuous explanatory variables such as plant biomass (shoot, root, total), soil moisture and soil N content were tested as covariables in the models for identifying potential mechanisms. We used Statistica 8 package (StatSoft Inc) for graphical presentation of data.

Results

Earthworm body weight. In total, 81 % of the 144 *Lumbricus terrestris* used were retrieved at the end of the experiment and on average, their biomass decreased by 32%. Neither the CO_2 nor plant species richness treatments had a significant effect on earthworm biomass (Table 1). They did, however, have a significant interactive effect (CO_2 x SR; Table 2) with earthworms exhibiting lower biomass in the eight species mixtures at ambient (-37.8%) than at elevated CO_2 (-29.5%) (Fig. 1a).

198 *Soil N.* Soil nitrogen content at the end of the experiment was not affected by the CO_2
199 treatment alone or by the presence of earthworms (Table 1). Increasing plant species richness
200 led to significantly lower soil N (Table 1), with the lowest N concentration in the eight
201 species mixtures (0.13% N) compared to one and four species mixtures (0.14% N).
202 *Soil C/N ratio*. Increasing plant diversity led to significantly higher C/N ratio (Fig. 1b).
203 Furthermore, the C/N ratio was affected by the interaction between CO_2 and earthworms
204 (CO_2 x EW) with the lowest C/N ratio in the presence of legumes under ambient CO_2
205 conditions.
206 *Soil moisture content.* Elevated CO_2 increased the soil moisture measured at the end of the
207 experiment from 25.3% in the ambient CO_2 to 29.3%. Increasing plant species diversity led
208 to significantly lower soil moisture in the four (26.1%) and eight (25.9%) species richness
209 compared to monocultures (30.1%, Table 1). Soil moisture was also affected by the
210 interaction between CO_2 concentration and the presence of earthworms (CO_2 x EW; Table 1
211 and Figure 1c), with significantly less available soil water in the earthworm treatment and
212 with the lowest amount of available soil water content at ambient CO_2 in the presence of
213 earthworms (Tukey HSD <0.05).
214 *Microbial biomass.* Elevated CO_2 marginally increased microbial biomass (+6.2%) (Table 1).
215 Plant species richness also affected the microbial biomass, with significantly higher biomass
216 in the four species richness (226.0 mg Cmic g^{-1} soil dry weight) compared to monocultures
217 (208.2 mg Cmic g^{-1} soil dry weight, Tukey HSD <0.05), with the eight species mixtures
218 being intermediate (217.5 mg Cmic g^{-1} soil dry weight, Tukey HSD >0.05). Additionally, the
219 CO_2 treatment, earthworm presence and plant species richness had an interactive effect (CO_2
220 × EW × SR; Table 2, Fig. 2) on the microbial biomass; under elevated CO_2 only four and
221 eight species mixtures increased microbial biomass (Tukeys HSD <0.05) compared to

monocultures if earthworms were present, while in the absence of earthworms microbial biomass was higher in the monocultures (Fig. 2).

Discussion

Despite their small scale and somewhat artificial nature, microcosm experiments have a good track record of providing an indication of processes and mechanisms directing ecosystem functioning at much larger scales (Benton, Solan, Travis & Sait 2007). Given that all microcosms were constructed by using homogenised soil with standardised plant litter and no additional litter entered the soil, we contend that the observed effects of elevated CO_2 were occurring through the rhizosphere. Although the experiment discussed in this paper lasted only for the equivalent of one vegetative season, the observations show several interactive effects of elevated CO_2, earthworm presence and plant diversity on the functioning of the belowground compartment of a model temperate grassland.

Earthworms

It is widely accepted that the main drivers through which elevated CO_2 will affect soil organisms are soil moisture (Niklaus et al. 2007) and plant derived organic matter inputs such as litter and rhizodeposition (O'Neill 1994, Meehan et al. 2010). Changes in both quantity and quality of soil organic matter inputs have been shown to change with increasing plant diversity, due to alterations in the amount and chemical composition of organic matter entering the soil. This may potentially have beneficial effects on the earthworm community (Edwards 1996; Milcu, Partsch, Scherber, Weisser & Scheu 2008) though the relationship between plant species diversity and earthworm performance is unlikely to be straightforward with idiosyncratic effects often reported (Wardle, Bonner, Barker, Yeates, Nicholson et al. 1999; Eisenhower, Milcu, Sabais, Bessler, Weigelt et al. 2009). We observed higher

247 earthworm biomass in microcosms with higher plant diversity (8 species), but only under
248 elevated CO_2 treatment. However, since soil moisture, plant shoot, root and total biomass,
249 when used as potential covariables in the statistical model, did not render the CO_2 × SR
250 interaction non-significant¸ we contend that the increase in earthworm biomass under
251 elevated CO_2 is not a result of changes in soil moisture or plant biomass. As no aboveground
252 inputs entered the system, the greater earthworm biomass could only have resulted from
253 additional belowground inputs. Elevated CO_2 is known to generally enhance N
254 rhizodeposition (Schultze & Merbach 2008) and N_2 fixation in legumes (Stephanie,
255 Fischinger, Hristozkova, Mainassara & Schultze 2009) leading to increased total
256 belowground N transport. This likely explains the higher biomass of *L. terrestris*, an anecic
257 litter feeding species which has been previously shown to be closely linked to N availability
258 (Milcu et al. 2008). Interestingly, the lowest biomass of earthworms was observed at the
259 highest plant diversity level under ambient CO_2. This is in contradiction with existing
260 literature which documents positive or idiosyncratic (Hedlund, Santa Regina , Van Der
261 Putten, Lepš, Diaz et al. 2003; Eisenhauer et al. 2009), but no detrimental effects of
262 increasing plant diversity on earthworm performance. The observed decrease in earthworm
263 biomass was likely caused by the high competition for N between plants and earthworms as
264 documented by the lower soil N and higher C/N ratio in the eight species diversity mixtures
265 in an already low-N soil (0.13% N) (Fig. 1b). Indeed fitting the C/N ratio in the model
266 renders the CO_2 × SR interaction non-significant ($P>0.05$) and increases the fitness of the
267 model as estimated by the AIC.
268
269 *Microbial biomass*
270 Like earthworms, soil microorganisms are often found to respond to elevated

CO_2 by changing their community composition, overall biomass and activity (De Graaff, Mavan Groenigen, Six, Hungate & Van Kessel 2006; Blagodatskaya, Blagodatsky, Dorodnikov & Kuzyakov 2010). The magnitude and the sign of the effect of elevated CO_2 on microorganisms is not very clear, with no effects (Holmes et al 2006), detrimental (Hungate et al. 1996) or idiosyncratic responses having been documented (Zak, Pregitzer, King & Holmes 2000; Kanerva, Palojärvi, Rämö & Manninen. 2008). Here we only found a marginally increased microbial carbon biomass (Cmic) in the elevated CO_2. Changes in litter inputs, root biomass and production, soil moisture and rhizodeposition have been previously identified to affect microbial biomass under elevated CO_2 (Zak 2000). However, whilst we could not identify the exact mechanism, it is worth noting that this increase was mediated by belowground effects of elevated CO_2 as no aboveground plant litter entered the soil. Moreover, we found significant changes in response to plant diversity. Higher Cmic at higher plant diversity levels have recently been reported in a 7 year experiment (Eisenhauer et al. 2009). In our microcosms, we found the eight and four plant species mixtures to have higher C*mic* than the monocultures. However, contrary to our expectations, the highest biomass was found in the four and not in the eight plant species mixtures. Higher C_{mic} at intermediate plant diversity levels have been previously found in early stages of experiments manipulating diversity as the effects of plants on the C_{mic} increased with time (Eisenhauer et al. 2009).

The presence of animal ecosystem engineers such as anecic earthworms also affects the functioning of the belowground systems and microbial biomass has been noted to increase due to accelerated incorporation of nutrient rich litter into the soil. Sheehan, Kirwan, Connolly & Bolger (2008) suggested that the positive effect of anecic species on microbial biomass was greatest with increased food supply. We found that microbial biomass varied with the presence of earthworms, plant species richness and CO_2 level; higher microbial biomass was found under elevated CO_2 at higher plant diversity levels when earthworms

were present, but also at the lower diversity level in the absence of earthworms. Given the inconclusiveness in observed effects, we could not identify the exact mechanism behind this interaction as none of the measured covariates (root biomass, total plant biomass, soil moisture or soil N content) affected the significance level of the interaction. This highlights the complexity of belowground C fluxes, it is possible that a longer-term experiment might be able to uncover such a mechanism by allowing the systems to stabilise and transient effects to lose their influence.

Soil moisture

Water availability frequently limits the functioning of the detritivore system (Swift, Heal & Anderson 1979; Austin 2002) with many soil decomposers migrating into deeper soil layers or to entering a dormant stage under water stress, with consequences for the functioning of the ecosystems. Water availability is essential for normal earthworm physiology due to their cutaneous mode of respiration and for maintaining their coelomic hydrostatic pressure at levels that allow locomotion (Lavelle 1988). Although highly dependent on soil water, earthworm activities (e.g. burrowing, casting, midden formation) can affect the soil water dynamics in a feedback loop through effects on soil physical properties. The mechanisms include changes in soil porosity and stability of organomineral aggregates. By burrowing and by the creation of organomineral casts, earthworms are generally thought to exert a beneficial influence on soil water availability through increased water infiltration and by improving soil water holding capacity (Edwards 2004). However, negative effects of earthworm activity on soil water retention have also been reported (Blouin, Lavelle & Laffray 2007) and there is increasing evidence that elevated CO_2 affects belowground communities also through improved plant and soil water status due to reduced evapotranspiration via reduced leaf conductance (Niklas et al 2007). This could play an important role in the functioning of the

ecosystems particularly in conditions of water shortage when burrows increases water drainage and evaporative water loss. Our results are in line with the findings of Blouin et al. (2007), showing a significant decrease in soil moisture content in the presence of *L. terrestris* via preferential water flow through the vertical burrows which can account to up to 10% of the water flow (Edwards, Shipitalo, Owens & Norton 1989). Given that in our experiment the soil water content was reduced by earthworms in both CO_2 treatments, but that a smaller reduction was observed under to elevated CO_2 (Fig. 1c), this suggests that both reduced soil water retention and increased water use efficiency (Wullschleger, Tschaplinski & Norby 2002) contribute to the observed effects. However, it might not be possible to extrapolate our this finding to filed conditions due to differences in drainage and mater movement between microcosms and undisturbed soil profile. Our observations indicate that deep vertical macropores created by anecic eartworms may modify the effect of elevated CO2 on soil water availability, the mechanism behind this interaction remains to be verified in the field.

Soil nitrogen

In an extensive meta-analysis considering the effects of elevated CO_2 on nutrient cycling De Graaff et al. (2006) found no effects on N mineralisation rates, but documented increased soil C inputs leading to increased soil C/N ratio. Although we found no significant effect of CO_2 on the soil N or C/N ratio, we observed a decrease of soil N and an increase in the soil C/N ratio with increasing plant diversity. These kind of effects have previously been reported (e.g. Oelmann,Wilcke, Temperton, Buchmann, Roscher et al 2007 and Roscher, Thein, Schmid & Scherer Lorenzen 2008) and they are attributed to a better usage of the total resources, whether they be nitrogen or water (i.e. increased complementarity). This mechanism contributes to the positive relationship between species richness and above-ground biomass production and is often paralleled by a decrease in

346 belowground available N due to increased efficiency in exploiting the available resource pool
347 but facilitating an increased accumulation of plant biomass as compared to low-diversity
348 communities (Roscher et al 2008). In support of this we found an increase of total plant
349 biomass with increasing diversity ($F_{2,270} = 6.6294$, $p = 0.002$).

350 Earthworm presence is known to increase N cycling and availability (Scheu 2003;
351 Edwards 2004), however, in this study the presence of earthworms did not have any effect on
352 the availability of soil N. In our experiment this is presumably related to the low resource
353 (litter) inputs in our system, but we acknowledge that by measuring only changes in total soil
354 N we could have missed potential changes in available N.

355

356 In conclusion, the findings of this experiment emphasize several interacting
357 mechanisms by which elevated CO_2 can modify the established relationship between plant
358 diversity, earthworms and microorganisms. The results confirm our over-arching hypothesis
359 that changes in belowground resource allocation, soil water and N availability mediated by
360 elevated CO_2 alter the relationship between plant diversity and the functioning of the
361 belowground compartment. Some of the observed interactions might be transient or
362 magnified by the nature of this microcosm experiment. However, these results confirm the
363 existence of several mechanisms which could determine the response and adaptation of
364 essential soil functions such as decomposition, nutrient cycling, carbon storage, water
365 filtration and primary productivity to elevated CO_2 at the ecosystem level. Such mechanisms
366 and interactions need further investigation in order to understand and predict the interactive
367 effects of climate change and diversity loss on ecosystem functioning and stability.

368

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Table 1. Output table of mix-effects models for the effect of CO_2 treatment (CO_2), earthworm presence (EW), plant species richness (SR) and their interactions on the belowground response variables. Excluded effects refer to the treatment factors which have been removed from the model in order to achieve minimum adequate models (Crawley 2007).

Belowground measurements																	
	Earthworm biomass				Soil moisture				Soil N				Soil C to N ratio				Microbial biomass		
	df	*F*	*P*		df	*F*	*P*		df	*F*	*P*		df	*F*	*P*		df	*F*	*P*
CO_2	1,6	0.32	0.591		1,6	**11.12**	**0.015**		1,6	0.1	0.947		**1,6**	**0.48**	**0.514**		1,6	4.69	0.073
EW	n.a	n.a	n.a		**1,273**	**108.7**	**<.001**		1,253	1.1	0.284		1,256	2.75	0.098		1,256	0.9	0.342
SR	2,101	1.61	0.205		**2,273**	**7.9**	**<.001**		**2,253**	**0.161**	**0.021**		**2,256**	**3.54**	**0.030**		**2,256**	**3.1**	**0.047**
CO_2 × EW	excl.	excl.	excl.		**1,273**	**9.7**	**0.002**		1,253	0.1	0.777		excl.	excl.	excl.		1,256	0.1	0.969
CO_2 × SR	**2,101**	**3.54**	**0.032**		excl.	excl.	excl.		excl.	excl.	excl.		2,256	1.59	0.206		2,256	1.77	0.172
CO_2 × EW × SR	excl.	excl.	excl.		excl.	excl.	excl.		excl.	excl.	excl.		excl.	excl.	excl.		**2,256**	**4.0**	**0.018**

Figure legends

Figure 1. Two-way interaction effects of plant species richness (SR) and atmospheric CO_2 treatment on earthworm mass loss (A), effects of SR on soil C/N ratio (B) and two-way interaction effects of SR and earthworm presence on soil moisture (C). Error bars represent ± SE.

Figure 2. Three-way interaction effects between plant **s**pecies richness (SR), atmopsperic CO_2 and earthworm presence on the carbon (glucose) induced microbial biomass (Cmic). Error bars represent ± SE

Figure 1

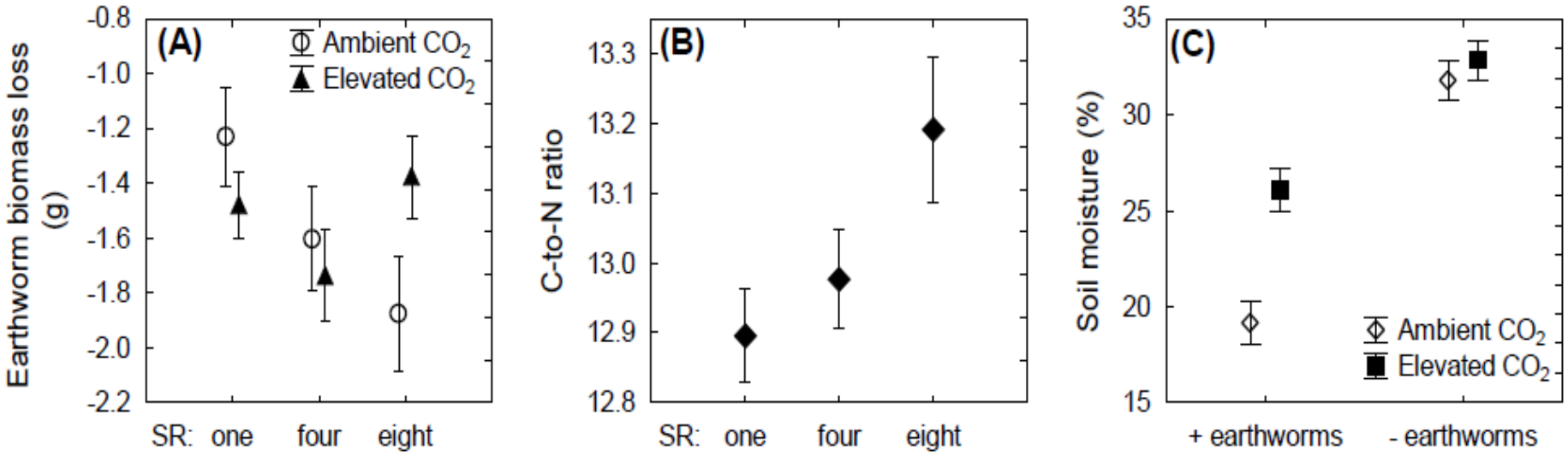
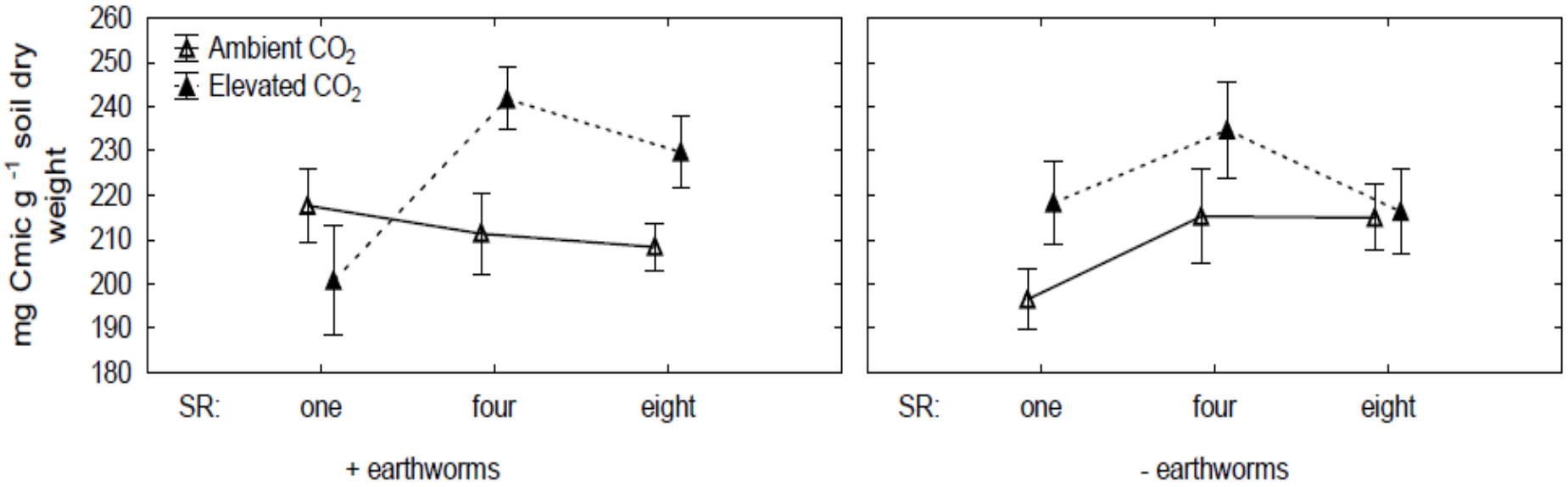

(A)
Earthworm biomass loss (g)
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
-2.2
Ambient CO2
Elevated CO2
SR: one four eight
(B)
C-to-N ratio
13.3
13.2
13.1
13.0
12.9
12.8
SR: one four eight
(C)
Soil moisture (%)
35
30
25
20
15
Ambient CO2
Elevated CO2
+ earthworms
- earthworms

Figure 2


mg Cmic g -1 soil dry weight
260
250
240
230
220
210
200
190
180
Ambient CO2
Elevated CO2
SR:
one
four
eight
+ earthworms
SR:
one
four
eight
- earthworms

Appendix A. Experimental design layout of the 18 different species mixtures. Grasses are shown in yellow, herbs in green and legumes in dark green.

Nr	Diversity level	Plant Species in Microcosm							
1	Monoculture	*Bromus hordeaceus*							
2	Monoculture	*Cynosurus cristatus*							
3	Monoculture	*Achillea millefolium*							
4	Monoculture	*Dipsacus fullonum*							
5	Monoculture	*Trifolium repens*							
6	Monoculture	*Vicia cracca*							
7	4-species-mixture	*Holcus lanatus*	*Lolium perenne*	*Vulpia bromoides*	*Festuca pratensis*				
8	4-species-mixture	*Taraxacum officinale*	*Rumex acetosella*	*Dipsacus fullonum*	*Daucus carota*				
9	4-species-mixture	*Trifolium dubium*	*Trifolium pratense*	*Medicago lupulina*	*Vicia sativa*				
10	4-species-mixture	*Bromus hordeaceus*	*Lolium perene*	*Leontodon hispidus*	*Vicia sativa*				
11	4-species-mixture	*Bromus sterilis*	*Plantago lanceolata*	*Taraxacum officinale*	*Lotus corniculatus*				
12	4-species-mixture	*Alopecurus pratensis*	*Myosotis arvensis*	*Trifolium pratense*	*Lathyrus tuberosus*				
13	8-species-mixture	*Holcus lanatus*	*Anthoxanthum odoratum*	*Festuca pratensis*	*Alopecurus pratensis*	*Agrostis capillaris*	*Festuca rubra*	*Bromus sterilis*	*Poa annua*
14	8-species-mixture	*Galium mollugo*	*Plantago lanceolata*	*Matricaria recutita*	*Daucus carota*	*Prunella vulgaris*	*Leontodon hispidus*	*Myosotis arvensis*	*Rumex acetosella*
15	8-species-mixture	*Trifolium dubium*	*Vicia sativa*	*Lathyrus pratensis*	*Vicia cracca*	*Lotus corniculatus*	*Medicago sativa*	*Trifolium medium*	*Medicago lupulina*
16	8-species-mixture	*Elytrigia repens*	*Anthoxanthum odoratum*	*Festuca rubra*	*Plantago lanceolata*	*Prunella vulgaris*	*Dipsacus fullonum*	*Medicago lupulina*	*Lotus corniculatus*
17	8-species-mixture	*Lolium perene*	*Bromus sterilis*	*Achillea millefolium*	*Matricaria recutita*	*Leucanthemum vulgare*	*Vicia sativa*	*Trifolium medium*	*Medicago sativa*
18	8-species-mixture	*Dactylis glomerata*	*Holcus lanatus*	*Festuca pratensis*	*Leucanthemum vulgare*	*Achillea millefolium*	*Trifolium dubium*	*Vicia cracca*	*Lathyrus tuberosus*

Grass
Herb
Legume