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Root responses to domestication, precipitation and silicification: weeping meadow grass simplifies and alters toughness

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Running title: Grass domestication impacts root traits

Keywords: deluge, drought, fracture strain, root architectural complexity, root trait selection, tensile strength

Abstract

Background and Aims

Plant breeding usually focuses on conspicuous above-ground plant traits, yet roots fundamentally underpin plant fitness. Roots show phenotypic plasticity in response to soil conditions but it is unclear whether domesticated plants respond like their ancestors. We aimed to determine how root traits differed between ancestral and domesticated types of a meadow grass (*Microlaena stipoides*) under altered regimes of precipitation and soil silicon availability.

Methods

We subjected the two grass types to three simulated precipitation regimes (ambient, +50%/deluge and −50%/drought) in soil with (Si+) and without (Si−) silicon supplementation and then characterised root biomass, architectural complexity and toughness in addition to shoot traits.

Results

Domestication increased root tissue density, decreased specific root length (SRL) and decreased root architectural complexity. Domestication also increased root strength under Si− conditions but not Si+ conditions. Fine roots, SRL, architectural complexity and the force required to tear the roots all decreased under deluge. The ancestral and domesticated grasses responded similarly to precipitation, except that the latter had weaker roots (decreased fracture strain) under drought.

Conclusions

Domestication and increased precipitation caused changes in *M. stipoides* root traits that could be beneficial against some stresses (e.g. soil compaction, herbivory) but not others (e.g. drought).

Abbreviations

RTD – root tissue density
SRL – specific root length
SRA – specific root area

ANC – ancestral

DOM – domesticated

Si – silicon

N – nitrogen

C – carbon

Introduction

Humans started to settle in communities about 10,000 years ago (Hillman et al. 2001), rapidly making hunter-gatherer societies obsolete (first evidence of agriculture may be as old as 32,000 years; Mariotti Lippi et al. 2015). To subsist, humans had to select, breed and crop wild cereals and legumes. Since then, plant domestication has artificially selected plants to increase their suitability to human requirements: taste, yield, and cultivation practices (Evans 1996; Smartt and Simmonds 1995). Domestication typically causes losses in genetic diversity through genetic drift and bottlenecks (Chen et al. 2015 and references therein) and has led to a suite of striking phenotypic changes, collectively referred to as the domestication syndrome (Harlan et al. 1973). Breeding objectives of the domestication process are framed within the agricultural or horticultural context and the environments in which plants will be used. Thus, the selection of traits of primary food crops will differ markedly from those of amenity (e.g. lawn) and forage grasses (Casler and van Santen 2010). The domestication of turf grasses, in particular, has generally targeted shoot traits such as colour, fine texture, seed quality and tiller density (Huyghe and Brummer 2013).

With the exception of a few species that have been domesticated for their edible roots, root traits are rarely the target of breeding programmes and have been neglected in domestication studies (Marshall et al. 2016). Given that more than 50% of net primary production is commonly allocated below-ground, sometimes approaching 90% (Coleman 1976), this limits our understanding of how domesticated plants will respond to biotic and abiotic stresses. Grasses, for example, commonly invest 50-70% of fixed carbon into the construction of roots, which are essential for water and nutrient uptake. Grass root architectural and physico-chemical traits are especially important for regeneration of above-ground parts after fire or herbivory (i.e. tolerance) and resistance against root herbivore attack, as well as for protection against soil compaction (i.e. the ability to maintain growth in compacted soils) and inadvertent uprooting by grazing ungulates (Gregory 2006; Moore and Johnson 2016).

Water availability can contribute significantly to changes in root architecture. Root traits associated with maintaining plant productivity under drought conditions, for example, include small fine root diameters and high root surface area, as well as long root lengths to access water deep in the soil profile (Comas et al. 2013). Mass-based specific root length (SRL), in particular, is often considered a measure of the ability of roots to proliferate in the soil and an indicator of environmental change (Ostonen et al. 2007). A high SRL (i.e. thin roots) tends to improve water and nutrient uptake efficiency. Similarly, measures of root complexity (i.e. root tip and branching density) and fine root volumes often reflect the absorptive potential of the root system (Børja et al. 2008).

Silicon (Si), an important element in plant nutrition, can mitigate various biotic and abiotic stresses, including herbivory and drought (Hartley and DeGabriel 2016; Ryalls et al. 2017). Root silicification, in particular, has been shown to increase resistance to drought, not by supporting root water retention capability, but by decreasing root growth inhibition imposed by desiccation (Soukup et al. 2017). Other studies, however, suggest that Si can alleviate osmotic stress by affecting stomatal aperture and preventing water from escaping through plant tissues (Debona et al. 2017 and references therein). Silicon has also been shown to promote ductility (Hattori et al. 2003) and increase root strength and soil penetration (Hansen et al. 1976; Lux et al. 2003), some of which are likely to increase water uptake. By understanding the effects of domestication on Si uptake and tolerance of water stress it may be possible to tailor future breeding practices that select for positive plant defence traits without sacrificing others (Simpson et al. 2017). One of the most important characteristics of Australian native grasses, for example, is drought tolerance, as they have evolved under either continuous or periodic drought (Malory 2014). Thus, maintaining drought-tolerant traits throughout the domestication process are of paramount importance, especially as the variability of rainfall increases (Fita et al. 2015).

Primary plant domestication has frequently selected for a simplification of plant architecture above-ground, with reduced branching and tillering (Chen et al. 2015 and references therein), as these changes in morphology result in higher yields. The greater tillering ability of their ancestors is often related to defence traits to tolerate herbivory, giving plants more opportunities and nodal points to compensate for herbivore damage (Chen and Romena 2008; Rosenthal and Welter 1995). Such simplification also results in a lower habitat complexity and can hinder natural enemies (Chen and Bernal 2011). Desirable characteristics of forage and lawn grass species, in contrast, tend to include high tiller numbers and high dry matter yield (Sokolović et al. 2014), which may improve tolerance of herbivory. Regardless of the intended use of different plant species, conspicuous above-ground plant traits may trade-off with inconspicuous below-ground root traits that are more important for resisting root- and shoot-feeding herbivores. Plant breeding efforts aimed at modifying root traits (e.g. using root phenotyping to identify desirable root characteristics) can result in novel, more stress-tolerant plants with higher yields and optimised water and nutrient acquisition, yet the responses of root morphology and defence traits to domestication remain largely unknown (Paez-Garcia et al. 2015).

Grass root architectural and physico-chemical traits are vital for protection against soil compaction, herbivory and resistance to abiotic stress (e.g. drought and deluge). This study investigated the impacts of water stress and Si application on the root architecture and physical traits of both ancestral and domesticated *Microlaena stipoides* (Labill.) that has been bred over the course of 10 years. We specifically aimed to understand how changes in root morphology can be used to improve plant performance and resist various biotic and abiotic stresses. There is an increasing realisation of the importance of Si to grass ecology and the majority of studies focus on Si deposition in aboveground plant parts despite the fact that Si can also be deposited as phytoliths in grass roots (Frew et al. 2016). The application of Si was incorporated to determine whether root Si concentrations would increase plant stress tolerance. Domestication is an example of rapid evolution by artificial selection and breeding, making it especially suitable for studying trait development on a contemporary timescale. The grasses (Poaceae) are by far the most economically-important plant family and many have been bred for pastures, lawns and cereal production (Glémin and Bataillon 2009). *Microlaena stipoides*, in particular, is among the most important native Australian species valued for its high pasture quality for livestock, beneficial architectural traits, large grain size and adaptability to a range of soil types and environments (Mitchell et al. 2014). It has been identified as having potential for commercial production as a new grain crop for human consumption, responding well to nitrogen application and regular irrigation (Malory et al. 2011). However, further understanding of key domestication traits of *M. stipoides* is required to aid the domestication process (Shapter et al. 2013). Beneficial root traits, in particular, that improve plant growth and increase tolerance to herbivory would be important to maintain. Ancestral plants may well express traits that should be reincorporated into domesticated plants. We hypothesised that the selection of *M. stipoides* aboveground physiological traits, including increased tillering and height (Huyghe and Brummer 2013) over the last 10 years would trade off with growth and architectural complexity below-ground due to a balancing metabolic cost for resource allocation (Paez-Garcia et al. 2015). This would simplify root architectural traits and make them more susceptible to soil compaction and uprooting by grazing ungulates. In other words, domestication was expected to shift resource allocation in favour of aboveground physiological traits, similar to that found in wheat (Qin et al. 2012). We expected breeding to favour drought-tolerant traits (e.g. increased root length and fine root volume to increase access and uptake of soil water), which would benefit further from increased Si uptake in Si-supplemented plants.

Materials and methods

To study the impact of domestication on root traits in *M. stipoides*, two sets of seeds from the variety Burra (obtained from Native Seeds, Cheltenham, VIC, Australia) were tested. Burra weeping grass is particularly suitable for lawn, pastures and revegetation. The first set (ancestral) was harvested in 2006 whereas the second set (domesticated) was harvested in 2015. Over 10 years, the domesticated type has been subject to selection for colour, persistence and seed production (full details in Mitchell (2013) and references therein).

Plant growth conditions

Yarramundi Loam (Hawkesbury Campus, Western Sydney University, NSW, Australia; see Online Resource 1, Table S1 for soil chemical composition) was sieved to <2 mm and placed in rhizotubes (4.5 × 4.5 × 50 cm), up to approximately 2 cm from the surface (800 g of dry soil (0.79 g cm^{-3}) per tube). Rhizotubes were constructed of square plastic cable trunk. One end of each section was sealed with weed control mat (Coolaroo®, Gale Pacific Inc., VIC, Australia). To ensure that water was not a limiting factor during germination, the rhizotubes were saturated with water for two days prior to planting. 300 seeds per grass type were individually sown in the rhizotubes (five per tube). Pots were then covered with cling film for one week to ensure adequate moisture for germination and establishment of the seedlings. After germination, seedlings were removed to keep only one individual per rhizotube.

Plants were grown in a glasshouse (25°C, 60% RH) in Richmond, NSW (latitude -33.611131, longitude 150.745354) with a natural source of light for 12 weeks (11 January 2016 – 28 March 2016; ca. 13:11 light:dark) and assigned, from one week after planting, at random to one of three simulated precipitation treatments (denoted as ‘precipitation’ for brevity), giving 40 plants per precipitation regime. One third of the plants were watered with 15 mL (ambient precipitation over 30 years in Richmond, NSW), one third with 7.5 mL (drought, 50% of ambient) and one third with 22.5 mL (deluge, 150% of ambient) three times per week with tap water. Silicon (Si) was applied to half of the plants by watering with 500 mg L^{-1} solution of dissolved sodium metasilicate ($Na_2SiO_3.9H_2O$) three times per week (drought-, ambient- and deluge-treated plants received 0 mL water + 7.5 mL Si, 7.5 mL water + 7.5 mL Si and 15 mL water + 7.5 mL Si, respectively). No nutrients were provided to the plant over the course of the experiment. Plant heights, from the base to the canopy, were measured weekly from four weeks after sowing until harvest. At week 12, the numbers of grass tillers were counted, the rhizotubes were disassembled and the soil was gently removed from the root systems. The roots were then washed from the remaining soil over 200 µm and 20 µm stacked sieves.

Root architectural properties

Immediately after washing, roots were immersed in water with 5% ethanol in a transparent tray and scanned with a flat-bed scanner (Expression 11000XL, Epson, NSW, Australia). The number of forks (i.e. root branching identified as points where three lines intersect) and tips (i.e. root endings), total root lengths, volumes and surface areas and average root diameters, as well as root volumes per diameter class, were determined by image analysis using WinRHIZO Pro 2015 (Regent Instruments Inc., QC, Canada) (Bouma et al. 2000). Roots were oven dried at 40 °C for three days and weighed. Total root dry mass and architectural traits were used to calculate specific root length (SRL; cm g^{-1} root dry mass), specific root area (SRA; cm^2 g^{-1} root dry mass), root tissue density (RTD; g cm^{-3} root volume), as well as specific root tip and branching density (tips g^{-1} root dry mass and forks g^{-1} root dry mass, respectively).

Root physical traits

Two physical traits of the roots of the ancestral and domesticated grasses were measured immediately after scanning (before drying). First the physical strength of the root was evaluated using a tearing apparatus adapted from Pérez-Harguindeguy et al. (2013) (Online Resource 1, Fig. S1). From each root system, the top 8 cm of the main root were cut with a razor blade and individually scanned and analyzed in WinRHIZO Pro 2015. The average diameter of the root was recorded. Both ends of the section of root were wrapped in Parafilm (Bemis Inc., WI, USA), to prevent compression damage to the root, while ensuring a good hold with the clamps of the tearing apparatus, placed ca. 3 cm apart. Roots were torn apart. The force to fracture the root was recorded using a digital force gauge (FPG-5, Test-Equip, VIC, Australia). Tearing force (F_t) was calculated as

$$F_t = \frac{Force\ to\ fracture}{Diameter\ of\ the\ tested\ root}$$

and expressed in N mm^{-1}.

In addition to the tearing force (otherwise referred to as tensile strength), the exact distance between the root tearer clamps was measured prior (D_p, when the root was just under tension) and immediately after (D_a) the fracture of the root. Root fracture strain, otherwise known as root elongation at break (E_r), a measure of ductility, was calculated as

$$E_r = \frac{Da - Dp}{Diameter\ of\ the\ tested\ root}$$

and expressed in mm mm^{-1}.

Root chemistry

Dried roots were ball-milled to a fine powder. Carbon and nitrogen concentrations were determined with a Carlo Erba CE1110 elemental analyser and Si concentrations were determined using an X-ray fluorescence spectrometer (Epsilon-3x, PANalytical, EA Almelo, The Netherlands) using small mass holders, as described in Hiltpold et al. (2016). Total phenolic concentrations in the roots were determined as described in Salminen and Karonen (2011), using a Folin-Ciocalteu assay with gallic acid monohydrate (Sigma-Aldrich, St. Louis, MO, USA) as the quantification standard.

Statistical analyses

The R statistical interface v3.3.3 (R Core Team 2017) was used for all statistical analyses. The effects of domestication, Si application and precipitation on plant growth (height, root biomass and number of tillers), root strength (F_t and E_r), root architecture (total length, surface area and volume, average diameter, RTD, SRA, SRL, root tip and branching density) and root chemistry (CN ratio, Si concentrations and total phenolic concentrations) were analysed using general linear models (ANOVA). Plant heights were analysed using a linear mixed model in the *nlme* statistical package (Pinheiro et al. 2017), with pot number and time (week number) as random effects to account for repeated measures. The fixed terms for all models included grass type (domesticated and ancestral), precipitation regime (drought, ambient and deluge) and Si application (Si- and Si+) as well as the two-way and three-way interactions between these terms. Post-hoc Tukey's tests using the *glht* function in the package *multcomp* (Hothorn et al. 2008) were used for pairwise comparisons of means for treatment and interaction effects. Where appropriate, dependent variables were transformed before analysis (Table 1). Correlations between phenolic concentrations, Si concentrations and root strength and architectural characteristics were determined using the function *cor.test* in the package *stats* (R Core Team 2017). Permutational analysis of variance (PERMANOVA) was used to model the multivariate relationship between total raw architectural traits and root mass using the package *vegan* (Oksanen et al. 2017). A principal component analysis (PCA) was performed to visualise significant treatment effects using the packages *stats* and *ggplot2* (Wickham 2009).

Results

Plant growth

Plant height significantly increased as simulated precipitation levels increased from drought to deluge, which became clear within eight weeks after sowing (Fig. 1). Domesticated plants grew significantly taller than ancestral plants overall (Table 1). Grass type and precipitation had significant effects on other plant growth characteristics. In particular, domestication increased tiller numbers and root biomass. These characteristics also increased as precipitation increased (Table 1). Si application had no significant effects on plant growth. Full model statistics, including two-way and three-way interaction effects between grass type, precipitation and Si-application, are shown in Table S2 (Online Resource 1).

Root architectural properties

Root biomass was significantly correlated with total root architectural traits (i.e. total volume, surface area and length) overall (PERMANOVA $F_{1,75} = 36.458$, $R^2 = 0.327$, $P < 0.001$). Accounting for changes in root biomass, domestication increased RTD but decreased SRL and SRA. Domestication had no significant effect on average root diameter. Precipitation significantly affected all architectural traits, whereby deluge increased average root diameter and RTD but decreased SRL and SRA compared with ambient precipitation (Table 1). The PCA output combining all architectural traits showed significant overlap between rainfall treatments (Online Resource 1, Fig. S2) and between ancestral and domesticated plants (Fig. 2). Direction of effects of PCA variables showed that RTD was positively correlated with root mass and negatively correlated with SRL and SRA (Fig. 2). SRL was closely associated with SRA. Architectural complexity traits (i.e. specific root tip and branching density), which were positively correlated, decreased in domesticated plants and under deluge compared with ancestral plants and ambient precipitation, respectively (Fig. 3a and b). The proportional volume of roots that were less than 0.4 mm diameter generally decreased with domestication and deluge (Fig. 3c and d, respectively; also see Table 1 and Online Resource 1, Table S2 for full statistics), showing similar patterns to changes in SRL (Fig. 3a and b) and root architectural complexity. Si application had no significant effects on root architectural properties (Online Resource 1, Table S2).

Root physical traits

Grass type and Si application interactively affected both fracture strain and tearing force required to break the roots. In particular, when no Si was applied to the soil, root fracture strain and tearing force was greater in domesticated compared with ancestral *M. stipoides* (Fig. 4a and c, respectively), whereas both physical traits showed opposite, albeit insignificant, responses to domestication when Si was applied to the soil. Grass type and precipitation had a significant interactive effect on fracture strain, whereby ancestral *M. stipoides* roots were more ductile than domesticated roots, but only under drought (Fig. 4b). Precipitation alone had a significant effect on root tearing force (Table 1), with plants under deluge requiring significantly less force to break the roots than those under drought and ambient conditions (Fig. 4d).

Root chemistry

There were no significant individual or interactive effects of grass type, precipitation or Si application on the concentrations of Si in the roots. Precipitation had a significant effect on root C:N, with lower C and higher N in roots of plants that were subjected to deluge compared with those subjected to drought and ambient precipitation treatments. Total root phenolics were significantly affected by the interaction between grass type and precipitation, whereby concentrations in ancestral plants were significantly higher than those in domesticated plants under ambient conditions but tended to be lower when plants were water-stressed (Online Resource 1, Fig. S3). However, changes in phenolic concentrations were not significantly associated with Si concentrations (r = -0.16, P = 0.173, df = 70) or root strength characteristics (fracture strain r = 0.22, P = 0.065, df = 72; tearing force r = 0.06, P = 0.612, df = 72).

Discussion

Domestication of an Australian native grass

Domestication of *Microlaena stipoides* clearly has an impact on plant growth and root architecture, affecting eight of the nine characteristics measured. After ca. 10 years of selection, domesticated *M. stipoides* grew taller and had more tillers compared with plants grown from ancestral germplasm, as expected. Higher tillering and growth were likely favourable traits for domesticated *M. stipoides*, which is generally used for lawns and pasture, compared with food crop species such as wheat and maize that required more compact growth habitat and less branching as positive traits associated with harvesting (Chen et al. 2015; Glémin and Bataillon 2009). The main effects of domestication on *M. stipoides* are visualised in Fig. 5, with numbers referring to relevant figures associated with each effect.

Domestication had clear impacts on root architecture overall, although domesticated plants showed surprisingly less homogeneity in architecture (i.e. PCA clustering) than we expected compared with ancestral plants. Domesticated *M. stipoides* also tended to have stronger and more ductile roots than its ancestral type, although this was only the case when soil was not supplemented with Si. High root strength can have positive impacts on the plants' defence against herbivory above- and below-ground (Johnson et al. 2010; Turcotte et al. 2014). Domesticated plants may therefore be less susceptible to root herbivore attack under natural conditions (i.e. no Si supplementation), potentially producing higher yields in the case of high herbivore pressure. Root strength may also impact the anchorage of grass in the ground (Ennos 1991), therefore domesticated *M. stipoides* may be less susceptible to uprooting by mammalian grazing above-ground. Selecting *M. stipoides* as a pasture species may have favoured a stronger and more ductile root system to increase resilience to grazing. In general, however, domesticated plants were less complex and had a lower relative volume of fine roots (0-0.4 mm diameter) than ancestral plants, which may impair the ability of *M. stipoides* to tolerate disturbance (e.g. uprooting and tearing of tillers). The extent to which a simpler root system will affect abiotic and/or biotic resistance and counteract the positive effects of increased root strength is uncertain but should be considered in breeding programmes that focus primarily on above-ground traits. Moreover, root architecture can have a significant impact on natural enemies of insect pests in the rhizosphere. For example, entomopathogenic nematodes (EPNs) move in the vicinity of the roots and, in the absence of cues, their ability to locate and kill their insect host decreases as root architectural complexity increases (Demarta et al. 2014). Therefore, more complex root systems could negatively impact higher trophic levels in the event of root herbivory.

When soils were supplemented with Si, the positive effects of domestication on root toughness (i.e. fracture strain and tearing force) were diminished, although this effect was not associated with an increase in root Si concentrations. In particular, there were no clear effects of Si application on root Si concentrations, although any stored silicon, which is generally taken up by the roots in the form of monosilicic acid and stored as phytoliths above-ground, may not be obvious by looking at concentrations in the roots of *M. stipoides* (Richmond and Sussman 2003). Other grass species have demonstrated high Si-accumulation capacities in the shoots (Cooke and Leishman 2011) and roots (Moore and Johnson 2016), which demonstrates the importance of classifying idiosyncratic uptake efficiencies across species. While we expected to see higher root Si concentrations in Si-supplemented roots, *Microlaena* species, in particular, tend to have high foliar Si concentrations compared with other native species (Power et al. 2016) so Si uptake may already be maximised in natural soil environments which would limit additional uptake by the roots (Deshmukh and Bélanger 2016). In general, root Si concentrations were not affected by domestication and are therefore unlikely to affect the ability of *M. stipoides* to withstand abiotic and biotic stresses, as demonstrated in cereal crops above-ground (Simpson et al. 2017). Crop systems tend to experience strong selection for faster growth through domestication, which may reduce the resources available for investment in defence. Simpson et al. (2017), in particular, used wild and modern cultivated cereals, including wheat, barley and maize, to demonstrate a general 10% reduction in Si-based defences through domestication, which correlated positively with leaf tensile strength (i.e. tearing force) and negatively with phenolic concentrations. However, the phenomenon of plants being 'disarmed by domestication' via a trade-off between defence investment and growth rate (Massei and Hartley 2000) has not been found by other studies (Turcotte et al. 2014; Whitehead et al. 2017). The use of dependent types of *M. stipoides* in the current study makes it difficult to draw generalisations and extrapolate these findings to all domesticated and ancestral types of this species. Future studies using multiple domesticated and ancestral types would provide further insight into how changes in root morphology can be used to improve plant performance.

Responses of Microlaena stipoides to water stress and its interaction with domestication

In general, deluge had higher impacts on the above- and below-ground growth characteristics of *M. stipoides*, which is known for its tolerance to drought (refer to Fig. 5 for a visual summary of the effects of deluge on *M. stipoides*). SRL decreased under the two extreme rainfall treatments, resulting from decreased investment in root length. Root depth represents the key trait for the exploration of stored water so a decrease in SRL would reduce their ability to explore soil at depth. In drought stressed plants, the decrease in SRL was likely caused by the inhibition in root growth imposed by low water availability, whereas roots of plants subjected to deluge may simply have not been stimulated due to the plentiful water supply. Plants can improve nutrient uptake by modulating root growth and architecture (Kiba and Krapp 2016). In this case, however, increases in SRA and SRL, associated with improved uptake and utilisation of water, did not coincide with increases in root N concentration. While we didn’t measure N uptake specifically, the concentration of N relative to C increased in plants subjected to deluge compared with those subjected to drought and ambient precipitation treatments, suggesting that plants were not N-limited (de Vries et al. 2016). In N-limited soils we may well see even larger differences in root architecture as the plant attempts to increase the total absorptive surface of the root system and direct growth towards nutrient-rich soil patches (Kiba and Krapp 2016). Moreover, plants under deluge had relatively fewer fine roots compared with those under drought and ambient precipitation, consistent with the importance of fine roots in maximising water uptake. The density of roots near the soil surface is one of the most important traits for exploiting soil moisture when plants rely on rainfall to acquire water during root system development (Tron et al. 2015). Thus, future studies using soil cores to analyse spatial soil metrics together with architectural data may provide a useful indication of water and nutrient uptake efficiency. Measuring the abundance of fine roots at different depths within a soil core, for example, would enable us to determine how quickly the plants will take advantage of surface water and soil nutrient application.

The increase in plant and root growth under deluge observed in the current study, while increasing the ability to compensate for root damage, can have negative impacts on root strength. The root tensile strength of woody plants tends to decrease as root moisture content increases (Hales and Miniat 2017; Yang et al. 2016), yet further studies are required to understand the effects of soil moisture on root tensile properties of grasses, which are likely to become increasingly important under future climate change scenarios associated with more extreme rainfall events and longer periods of drought (Chiew et al. 2011; IPCC 2007). Grass type and precipitation interacted to affect root fracture strain, with domestication decreasing root fracture strain under drought conditions. If domesticated plants are weaker and less ductile under drought they may be more susceptible to root damage and uprooting. Indeed, the lower root complexity and relative volume of fine roots in response to domestication suggests that domesticated plants are less efficient at finding and taking up water and/or nutrients from the soil (Mazzacavallo and Kulmatiski 2015). Domesticated plants may have been selected for optimal performance in irrigated systems, whereas ancestral plants are not and so might be better adapted to water-stressed environments. This could create problems for the management of these grasslands by reducing productivity as the length of drought periods increase (Chiew et al. 2011; Fita et al. 2015). Precipitation did not interact with grass type to affect the tearing force required to break the roots, however, demonstrating the difficulty in finding a reliable metric for phenotyping root traits such as root physical strength.

The negative impact of domestication on total phenolic concentrations was counteracted by drought and deluge but this was not associated with Si concentrations, plant strength or growth traits. The impacts of Si on constitutive carbon-based plant defences have been variable (Fleck et al. 2015) and more studies are required to draw general trends on the impact of Si on carbon-based plant defences, especially in the roots (Frew et al. 2016). While shoot herbivory is often the selective force for agricultural domestication, root defence compounds are important to consider as determinants of root strength and resistance to herbivores. Decreases in root phenolic concentrations, for example, have the potential to increase root herbivore load and limit aboveground plant performance, strength and folivore resistance (War et al. 2012) Moreover, aboveground herbivory has been shown to alter root phenolic concentrations, which may further interact with changes in water availability (Erb et al. 2015; Piwowarczyk et al. 2017). Water stress in general decreased phenolic concentrations in ancestral plants but had no effect on domesticated plants. Phenolic concentrations tend to be positively associated with resistance to herbivory so it could be envisaged that water-stressed ancestral plants were more susceptible to herbivory compared with domesticated plants that experience water stress. However, there are several examples of positive relationships between root herbivore performance and root phenolic concentrations that may be associated with alternative defence mechanisms (Frew et al. 2016; Johnson and Nielsen 2012). In general, plant defence theory (e.g. growth-differentiation balance hypothesis) predicts that increased plant productivity would result in decreased plant defences but the allocation to defences in domesticated plants and their ancestors or wild relatives may not be limited by allocation trade-offs, as demonstrated by Turcotte et al. (2014).

Conclusions

Results show that breeding domesticated *M. stipoides* over a 10 year period has increased its RTD, rate of growth and tillering ability but decreased its SRL, SRA and fine root volume. Moreover, domestication consistently increased the strength of *M. stipoides* roots under control conditions (without Si addition), which may increase their resistance to soil compaction (Striker et al. 2006), herbivory or other below-ground stress factors (Moore and Johnson 2016). However, decreases in below-ground complexity may show opposite effects by limiting the plants' ability to resist biotic stresses, especially under high water availability when roots are less ductile and less complex. The main findings are summarised in Fig. 5. This study clearly demonstrates how the selection and breeding of pasture plants for certain traits can result in the unexpected selection of other traits, which may have both positive and negative consequences on herbivore population dynamics, especially for invertebrate communities that rely on the structural properties of the roots to survive. Understanding how root architectural and physical traits increase plant productivity and improve stress tolerance would provide tangible targets for breeders to select individuals with beneficial root phenotypes for enhanced plant performance.

Accessibility

Data deposited in the Dryad Digital Repository: doi: to follow

Supplementary material available in Online Resource 1:

Table S1 Chemical composition of Yarramundi loam soil used in experimental study.

Table S2 Plant growth, strength, architecture and chemistry responses to domestication (grass type), silicon (Si) application and precipitation treatments, including their two-way and three-way interactions from general linear models and mixed-effect models.

Fig. S1 Device used to measure the tensile force required to tear the roots of *Microlaena stipoides*.

Fig. S3 The interactive effects of precipitation and domestication on total phenolic concentrations.

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Tables

Table 1 Plant growth, root architecture and chemical responses to domestication (ancestral and domesticated *Microlaena stipoides*) and simulated precipitation treatments.

Response variable	**Grass type**		**Precipitation**		
	Ancestral	**Domesticated**	**Drought**	**Ambient**	**Deluge**
Plant growth					
Average height (cm)[a]	8.17 ± 0.41	10.85 ± 0.34	8.45 ± 0.40^{a}	9.40 ± 0.40^{a}	11.88 ± 0.56^{b}
	$F_{1,87}$ = 18.66, P < **0.001↑**		$F_{2,87}$ = 7.00, P = **0.002↑**		
Root biomass (g)[a]	9.61 ± 1.84	21.38 ± 2.72	9.39 ± 2.13^{a}	13.29 ± 3.01^{a}	29.60 ± 3.55^{b}
	$F_{1,67}$ = 11.11, P = **0.001↑**		$F_{2,67}$ = 14.19, P < **0.001↑**		
Number of tillers	8.14 ± 0.67	10.13 ± 0.52	7.12 ± 0.66^{a}	9.09 ± 0.64^{b}	12.24 ± 0.57^{c}
	$F_{1,73}$ = 7.56, P = **0.008↑**		$F_{2,73}$ = 16.14, P < **0.001↑**		
Root architecture					
Average diameter (µm)	1.87 ± 0.08	2.01 ± 0.06	1.74 ± 0.05^{a}	1.88 ± 0.08^{a}	2.28 ± 0.07^{b}
	$F_{1,74}$ = 2.67, P = 0.106		$F_{2,74}$ = 14.17, P < **0.001↑**		
Total volume (cm^{-3})	0.09 ± 0.01	0.16 ± 0.02	0.08 ± 0.01^{a}	0.10 ± 0.02^{a}	0.24 ± 0.02^{b}
	$F_{1,74}$ = 13.04, P < **0.001↑**		$F_{2,74}$ = 23.73, P < **0.001↑**		
Root tissue density[b] (RTD; g cm^{-3})	85.57 ± 7.94	110.99 ± 7.21	100.21 ± 9.03^{a}	89.04 ± 10.79^{a}	120.01 ± 6.59^{b}
	$F_{1,66}$ = 6.11, P = **0.016↑**		$F_{2,66}$ = 3.69, P = **0.030↑**		
Specific root area[a] (SRA; cm^{2} g^{-1})	4.90 ± 1.16	2.43 ± 0.24	2.75 ± 0.25^{ab}	5.05 ± 1.06^{a}	1.61 ± 0.14^{b}
	$F_{1,66}$ = 6.97, P = **0.010↓**		$F_{2,66}$ = 6.96, P = **0.002↓**		
Specific branching density (number of forks g^{-1})	48.52 ± 10.90	24.96 ± 5.93	33.41 ± 7.96^{ab}	51.49 ± 11.86^{a}	7.37 ± 2.17^{b}
	$F_{1,54}$ = 4.26, P = **0.044↓**		$F_{2,54}$ = 6.22, P = **0.004↓**		
Root chemistry					
Si concentration (%)	1.64 ± 0.12	1.72 ± 0.06	1.81 ± 0.10^{a}	1.54 ± 0.10^{a}	1.78 ± 0.10^{a}
	$F_{1,65}$ = 0.38, P = 0.540		$F_{2,65}$ = 2.19, P = 0.120		
CN ratio[a]	31.79 ± 1.29	32.32 ± 1.26	34.00 ± 1.53^{a}	35.00 ± 2.25^{a}	28.56 ± 0.68^{b}
	$F_{1,49}$ = 0.01, P = 0.906		$F_{2,49}$ = 6.82, P = **0.002↓**		
Phenolics (GAE mg g^{-1})[b]	19.37 ± 4.21	16.34 ± 1.31	17.65 ± 1.91^{ab}	23.23 ± 3.65^{a}	10.37 ± 1.19^{b}
	$F_{1,60}$ = 0.03, P = 0.855		$F_{2,60}$ = 4.13, P = **0.021↓**		

Overall means (± SE) and statistics for treatment effects shown. *P*-values highlighted in bold indicate significance ($P < 0.05$). Where appropriate, response variables were transformed ([a]Log, [b]sqrt) before analysis. Values with the same letters between precipitation treatments were not significantly different ($P < 0.05$) according to post hoc tests performed on general linear and mixed-effect models. Full model statistics, including interaction effects, are shown in Table S2 (Online Resource 1). Arrows indicate direction of the effect of domestication and deluge for grass type and precipitation treatments, respectively. Additional root architectural (specific root length and specific root tip density) and strength (fracture strain and tearing force) traits are displayed in Figs. 3 and 4, respectively.

Figure legends

Fig. 1 The effects of domestication and precipitation treatments on plant height over eight weeks. Acronyms denote ancestral (ANC) and domesticated (DOM) grass types. Values of bars are means (±SE).

Fig. 2 Principal component analysis of root architecture data (N = 86) with attribute loadings on the first two components PC 1 and PC 2. Plots and ellipses (representing 68% of the predicted data) are coloured according to grass type. Acronyms denote total length (Length), total surface area (SurfArea), total volume (Volume), dry mass (Mass), average diameter (Diameter), root tissue density (RTD), specific root length (SRL), specific root area (SRA), number of root tips g^{-1} dry mass (Tips) and number of forks g^{-1} dry mass (Branches)

Fig. 3 Root complexity (number of root tips g^{-1} dry mass) and specific root length (SRL) responses to domestication (a) and precipitation treatments (b). The relative (i.e. proportion of total) volume (± SE) of roots of different diameter classes in response to domestication and precipitation treatments are shown in C and D, respectively

Fig. 4 Mean (±SE) root fracture strain (a and b) and tearing force (c and d) applied to domesticated (white bars) and ancestral (grey bars) plants under different Si application and precipitation treatments. Bars with the same letters were not significantly different ($P < 0.05$)

Fig. 5 Summary of key results illustrating the effects of domestication (right) and deluge (left) on the architectural and root toughness traits of *Microlaena stipoides*. Red arrows indicate negative impacts, blue arrows represent positive impacts and black arrows represent null impacts. Dashed lines indicate interactive effects (i.e. effects dependent upon other contributing factors). Numbers refer to relevant figures associated with each effect. Acronyms denote root tissue density (RTD), specific root length (SRL), number of root tips g^{-1} dry mass (Root tips) and number of forks g^{-1} dry mass (Branching)

Fig. 1

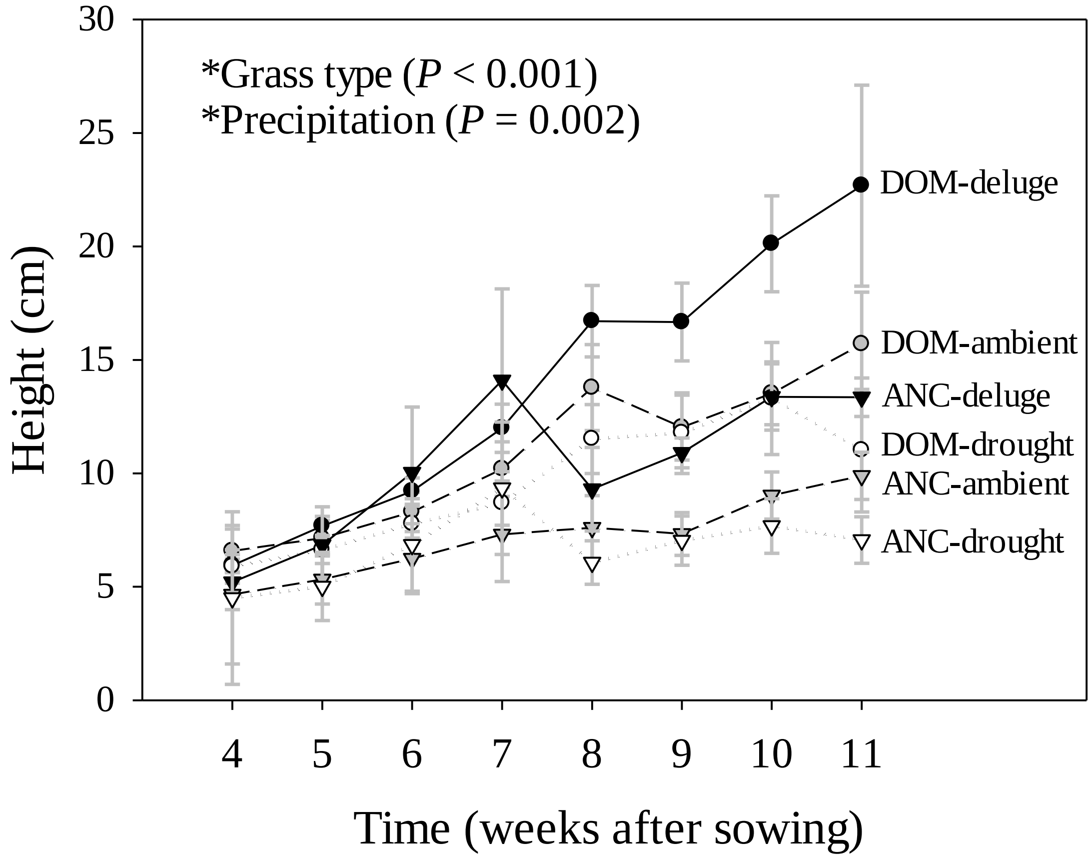
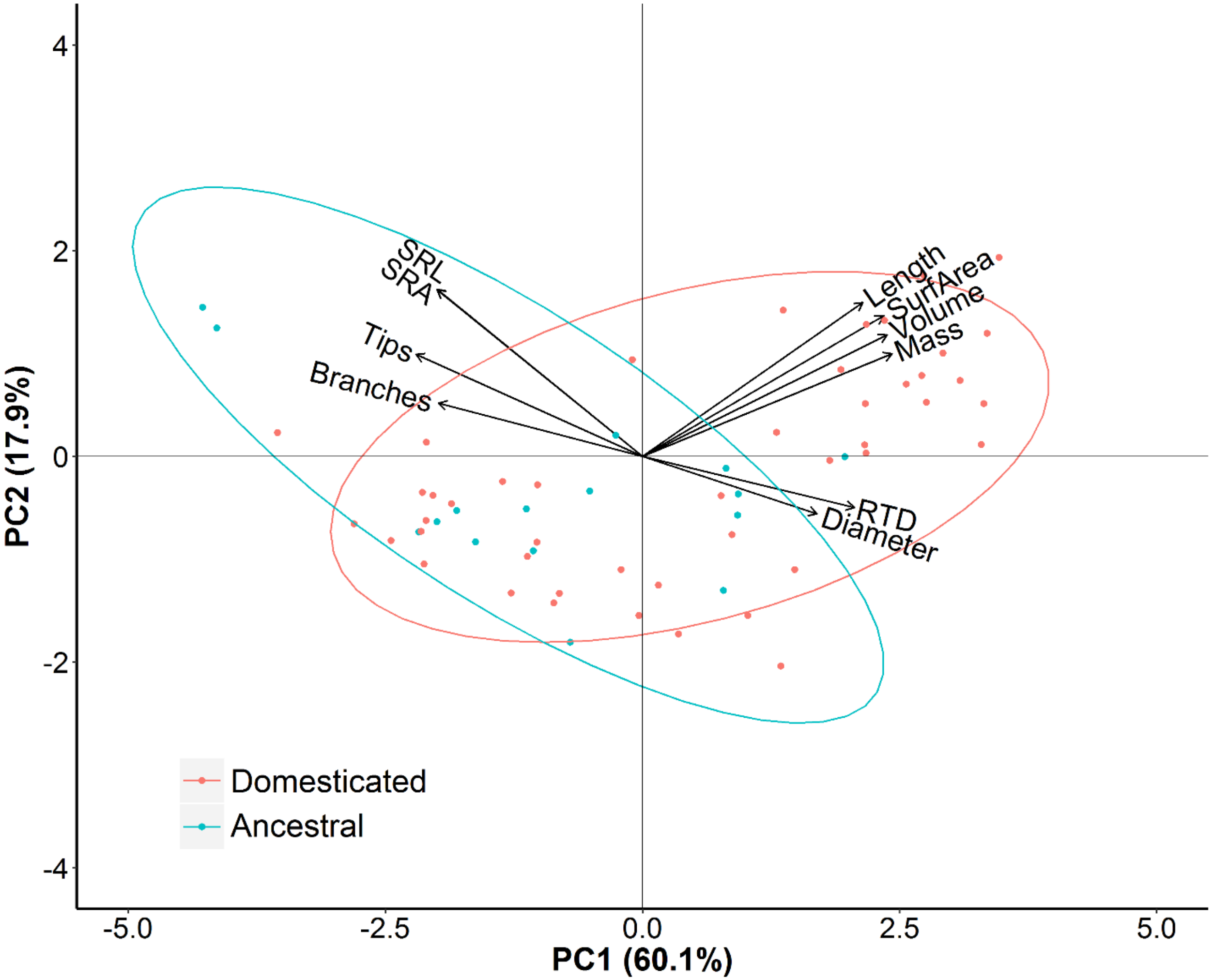

*Grass type (*P* < 0.001)
*Precipitation (*P* = 0.002)
DOM-deluge
DOM-ambient
ANC-deluge
DOM-drought
ANC-ambient
ANC-drought
Height (cm)
0
5
10
15
20
25
30
4
5
6
7
8
9
10
11
Time (weeks after sowing)

Fig. 2



4
2
0
-2
-4
PC2 (17.9%)
-5.0
-2.5
0.0
2.5
5.0
PC1 (60.1%)
SRL
SRA
Tips
Branches
Length
SurfArea
Volume
Mass
RTD
Diameter
Domesticated
Ancestral

Fig. 3

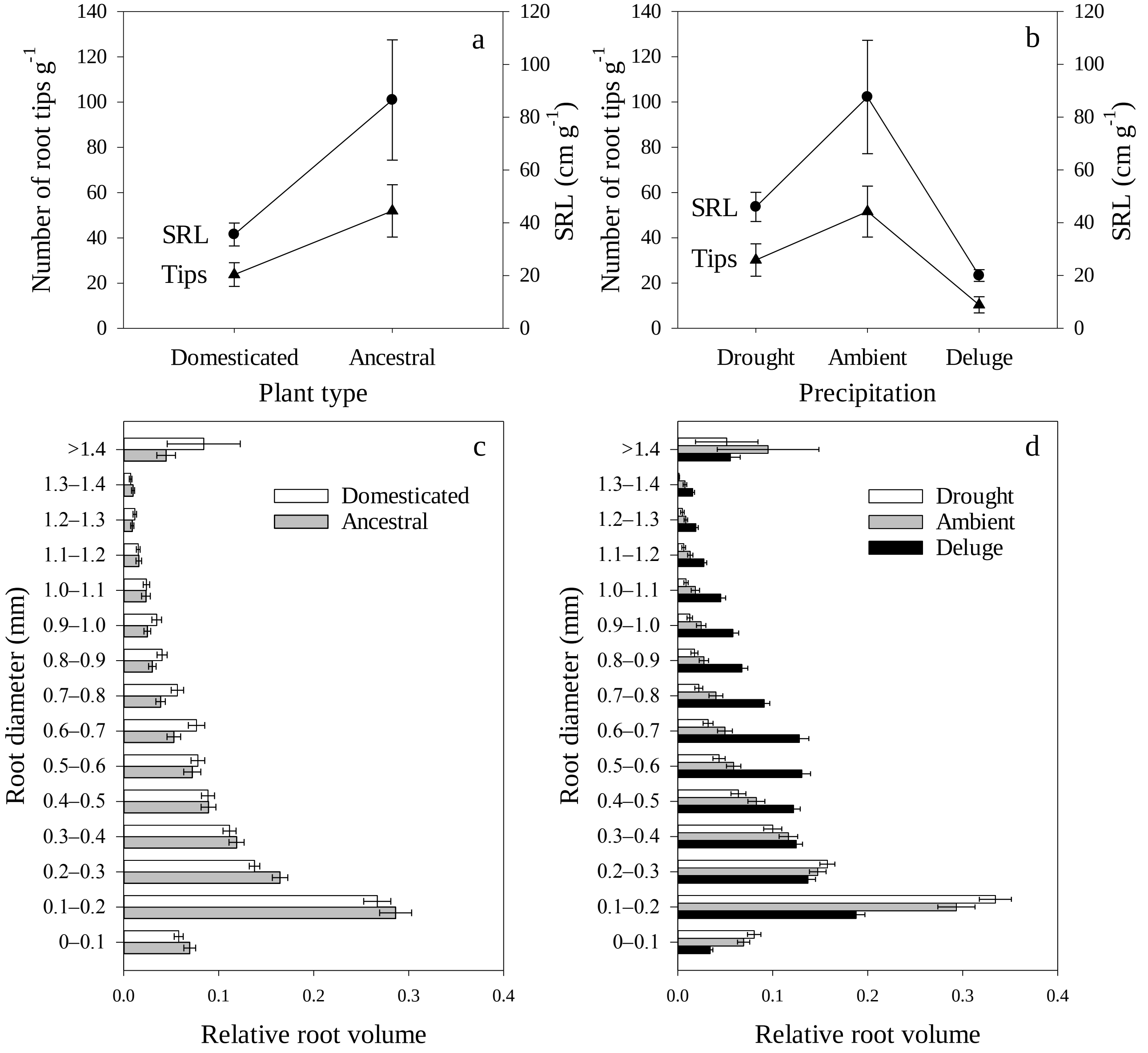

a
b
c
d
Number of root tips g^{-1}
SRL (cm g^{-1})
SRL
Tips
0
20
40
60
80
100
120
140
Domesticated
Ancestral
Plant type
Drought
Ambient
Deluge
Precipitation
Root diameter (mm)
>1.4
1.3–1.4
1.2–1.3
1.1–1.2
1.0–1.1
0.9–1.0
0.8–0.9
0.7–0.8
0.6–0.7
0.5–0.6
0.4–0.5
0.3–0.4
0.2–0.3
0.1–0.2
0–0.1
0.0
0.1
0.2
0.3
0.4
Relative root volume

Fig. 4

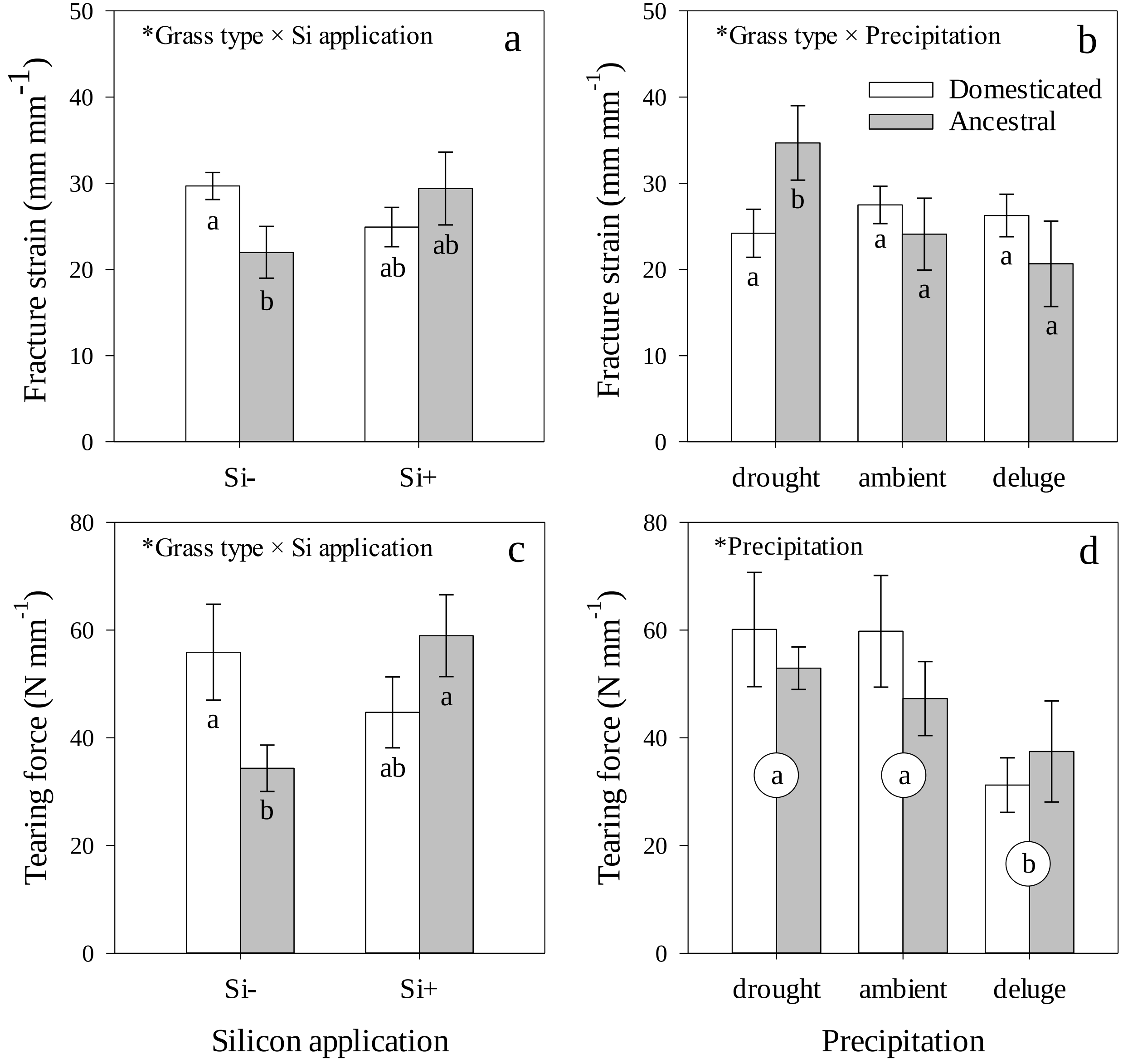
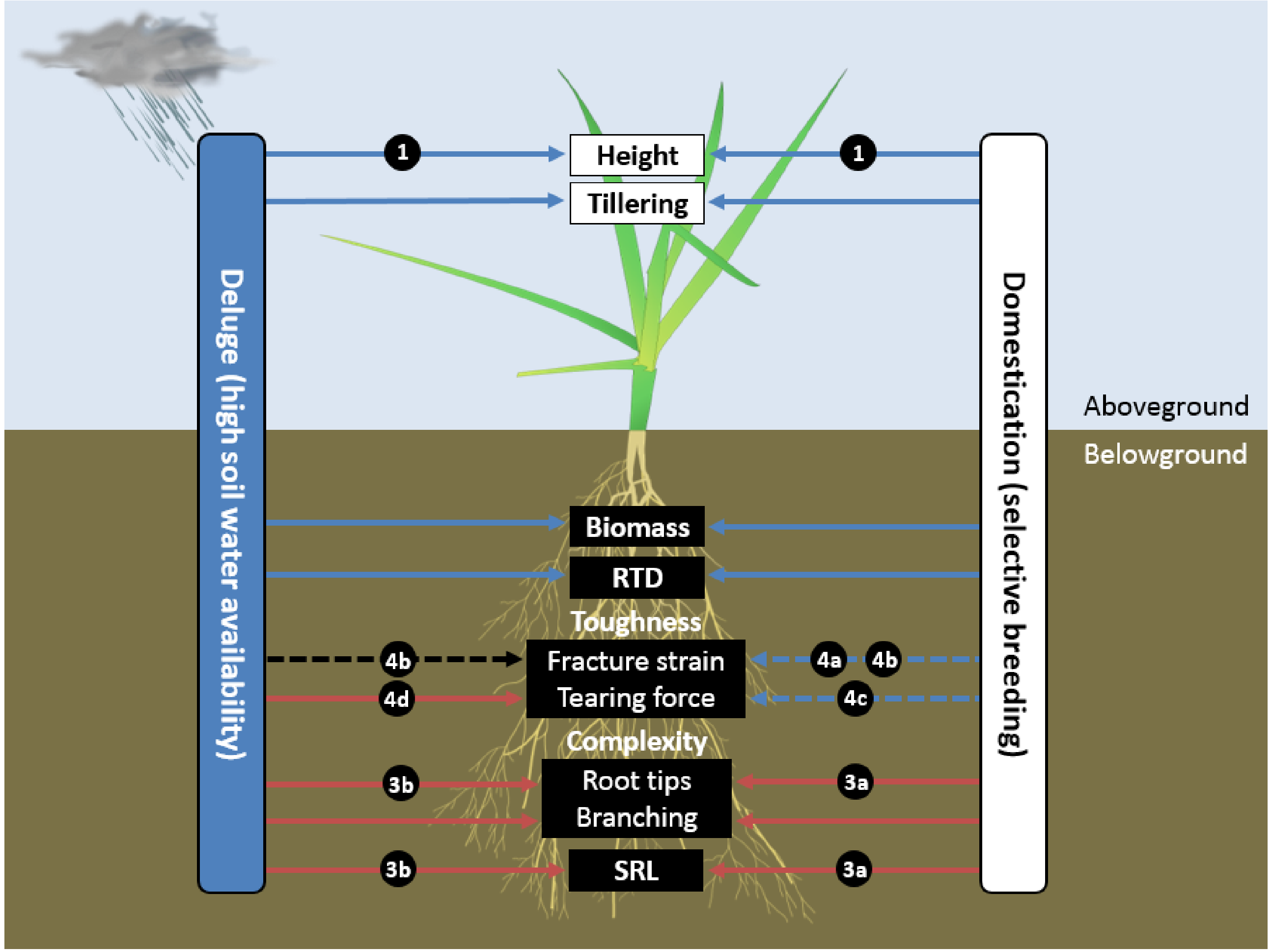

*Grass type × Si application
a
Fracture strain (mm mm^{-1})
50
40
30
20
10
0
a
b
ab
ab
Si-
Si+
*Grass type × Precipitation
b
Domesticated
Ancestral
Fracture strain (mm mm^{-1})
50
40
30
20
10
0
a
b
a
a
a
a
drought
ambient
deluge
*Grass type × Si application
c
Tearing force (N mm^{-1})
80
60
40
20
0
a
b
ab
a
Si-
Si+
Silicon application
*Precipitation
d
Tearing force (N mm^{-1})
80
60
40
20
0
a
a
b
drought
ambient
deluge
Precipitation

Fig. 5


Deluge (high soil water availability)
Domestication (selective breeding)
Height
Tillering
1
1
Aboveground
Belowground
Biomass
RTD
Toughness
Fracture strain
Tearing force
4b
4d
4a
4b
4c
Complexity
Root tips
Branching
3b
3a
SRL
3b
3a

Online Resource 1 – Supplementary material

Root responses to domestication, precipitation and silicification: weeping meadow grass simplifies and alters toughness

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Table S1 Chemical composition of Yarramundi loam soil used in experimental study.

Method	Nutrient / Property	Units	Mean	Standard Error
Colwell	Phosphorus	mg/kg	17.38	1.43
KCl	Nitrate Nitrogen	mg/kg	16.74	13.29
	Ammonium Nitrogen		2.75	0.37
	Sulfur		4.82	1.30
1:5 Water	pH	units	6.12	0.24
	Conductivity	dS/m	0.05	0.02
Calculation	Estimated Organic Matter	% OM	2.66	0.71
Ammonium Acetate + Calculations	Calcium	$cmol^+/Kg$	3.46	0.81
		kg/ha	1552.04	364.26
		mg/kg	692.87	162.62
	Magnesium	$cmol^+/Kg$	0.78	0.10
		kg/ha	213.13	26.62
		mg/kg	95.15	11.88
	Potassium	$cmol^+/Kg$	0.42	0.05
		kg/ha	369.84	41.43
		mg/kg	165.11	18.50
	Sodium	$cmol^+/Kg$	0.09	0.02
		kg/ha	44.91	8.53
		mg/kg	20.05	3.81
KCl	Aluminium	$cmol^+/Kg$	0.02	0.00
		kg/ha	3.66	0.62
		mg/kg	1.63	0.28
Acidity Titration	Hydrogen	$cmol^+/Kg$	0.05	0.01
		kg/ha	1.18	0.27
		mg/kg	0.53	0.12
Calculation	Effective Cation Exchange Capacity (ECEC)	$cmol^+/Kg$	4.82	0.89
Base Saturation Calculations	Calcium	%	70.46	3.55
	Magnesium		16.70	1.63

	Potassium		9.30	1.65
	Sodium - ESP		1.96	0.61
	Aluminium		0.41	0.12
	Hydrogen		1.17	0.34
Calculation	Calcium / Magnesium Ratio	ratio	4.35	0.69
$CaCl_2$	Soil pH ($CaCl_2$)	units	5.50	0.22
DTPA	Zinc	mg/kg	2.36	0.74
	Manganese		87.45	22.77
	Iron		70.46	5.00
	Copper		1.00	0.22
$CaCl_2$	Boron	mg/kg	0.29	0.03
	Silicon		23.42	0.83
LECO IR Analyser	Total Carbon	%	1.52	0.41
	Total Nitrogen	%	0.12	0.03
Calculation	Carbon/ Nitrogen Ratio	ratio	13.26	1.61

Table S2 Plant growth, strength, architecture and chemistry responses to domestication (grass type), silicon (Si) application and precipitation treatments, including their two-way and three-way interactions from general linear models and mixed-effect models.

Response variable	**df**	**Grass type**		**Precipitation**		**Si application**		**Grass type × Precipitation**		**Grass type × Si application**		**Precipitation × Si application**		**Grass type × Precipitation × Si application**	
		F_1	***P***	F_2	***P***	F_1	***P***	F_2	***P***	F_1	***P***	F_2	***P***	F_2	***P***
Plant growth															
Height*	87	18.66	**< 0.001**	7.00	**0.002**	0.37	0.543	0.16	0.854	2.66	0.107	0.93	0.398	2.53	0.086
Root biomass*	66	11.11	**0.001**	14.19	**< 0.001**	0.13	0.715	0.01	0.994	3.12	0.082	0.12	0.884	1.55	0.221
Number of tillers	72	7.56	**0.008**	16.14	**< 0.001**	1.57	0.215	0.65	0.524	0.28	0.599	1.65	0.199	3.06	0.053
Root architecture															
Total length*	74	6.51	**0.013**	12.53	**< 0.001**	1.17	0.283	0.75	0.478	2.68	0.106	1.81	0.170	3.02	0.055
Total surface area*	74	8.05	**0.006**	18.55	**< 0.001**	0.72	0.399	0.57	0.568	2.72	0.103	1.46	0.239	2.99	0.057
Average diameter	74	2.67	0.106	14.17	**< 0.001**	0.13	0.719	0.10	0.910	0.14	0.714	0.15	0.862	0.90	0.413

Total volume	74	13.04	**< 0.001**	23.73	**< 0.001**	0.01	0.910	1.63	0.203	1.04	0.310	1.41	0.252	2.27	0.111
Rel. fine root vol	74	7.09	**0.009**	20.82	**< 0.001**	1.60	0.211	0.36	0.698	0.08	0.775	1.69	0.191	2.91	0.061
Root tissue density#	66	6.11	**0.016**	3.69	**0.030**	0.79	0.376	0.91	0.406	3.78	0.056	0.61	0.547	0.96	0.389
Specific root length*	66	7.24	**0.009**	7.99	**< 0.001**	1.22	0.274	1.32	0.274	3.53	0.065	0.17	0.842	0.72	0.489
Specific root area*	66	6.97	**0.010**	6.96	**0.002**	1.60	0.210	1.29	0.282	3.61	0.062	0.34	0.711	0.69	0.504
Specific tip density	54	6.99	**0.011**	6.02	**0.004**	1.23	0.272	0.17	0.842	2.66	0.108	0.01	0.994	0.08	0.922
Specific fork density	54	4.26	**0.044**	6.22	**0.004**	0.62	0.434	0.14	0.872	1.56	0.216	0.01	0.995	0.49	0.617
Root strength															
Fracture strain#	71	0.11	0.737	0.47	0.626	0.16	0.687	3.88	**0.025**	5.30	**0.024**	1.87	0.162	2.28	0.110
Tearing force*	72	0.18	0.666	3.60	**0.032**	0.07	0.789	0.94	0.395	4.63	**0.035**	1.12	0.331	0.02	0.982
Chemistry															
Si concentration	64	0.38	0.540	2.19	0.120	0.04	0.845	2.44	0.095	0.20	0.652	1.23	0.298	1.57	0.215
CN ratio*	48	0.01	0.906	6.82	**0.002**	0.66	0.421	0.83	0.441	3.63	0.063	1.90	0.161	1.45	0.244
Phenolics#	59	0.03	0.855	4.13	**0.021**	3.71	0.059	8.20	**0.001**	0.01	0.936	2.91	0.062	0.94	0.396

P-values highlighted in bold indicate significance ($P < 0.05$). Where appropriate, response variables were transformed (*Log, $^{\#}$sqrt) before analysis.

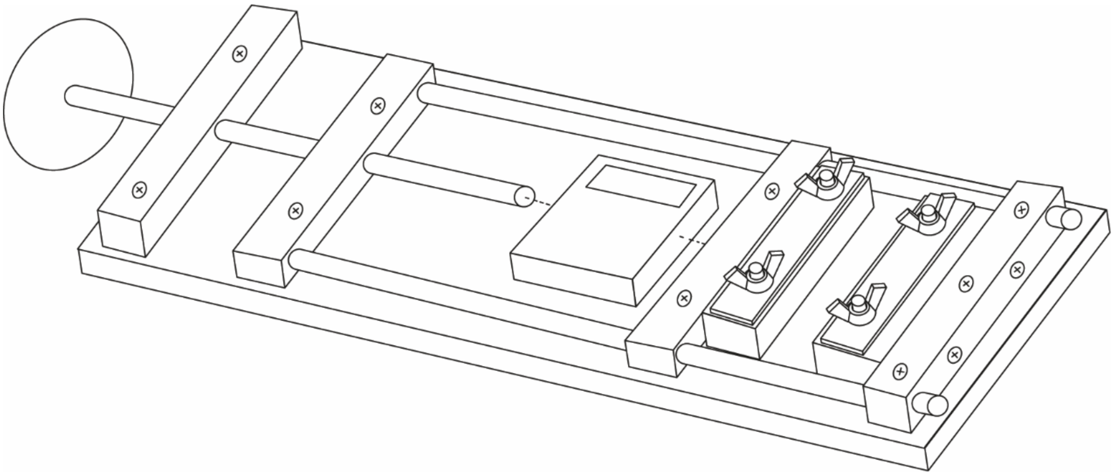

Fig. S1 Device used to measure the tensile force required to tear the roots of *Microlaena stipoides*.

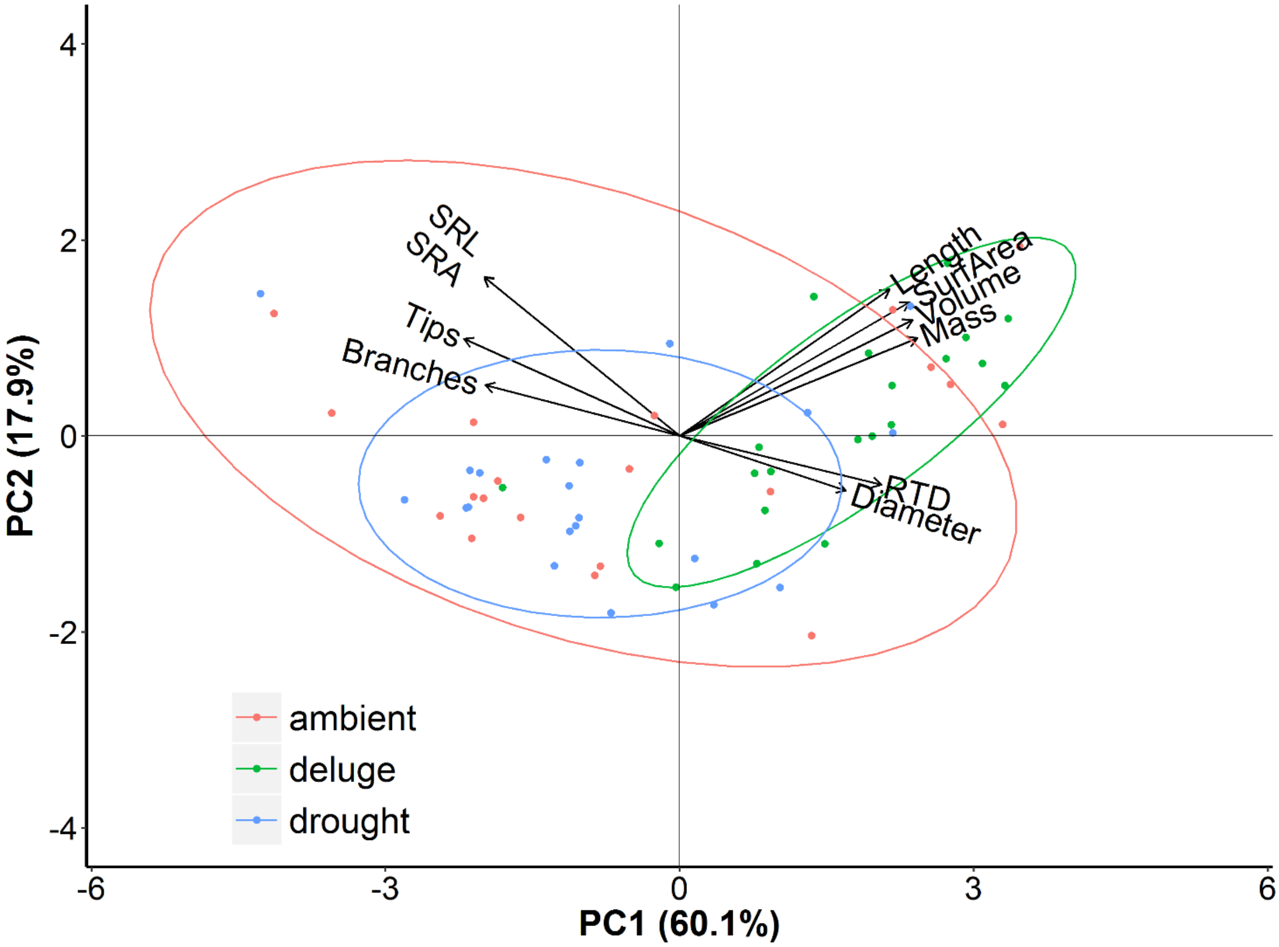

Fig. S2 Principal component analysis of root architecture data (N = 86) with attribute loadings on the first two components PC 1 and PC 2. Plots and ellipses (representing 68% of the predicted data) are coloured according to rainfall treatment. Acronyms denote total length (Length), total surface area (SurfArea), total volume (Volume), dry mass (Mass), average diameter (Diameter), root tissue density (RTD), specific root length (SRL), specific root area (SRA), number of root tips g^{-1} dry mass (Tips) and number of forks g^{-1} dry mass (Branches)

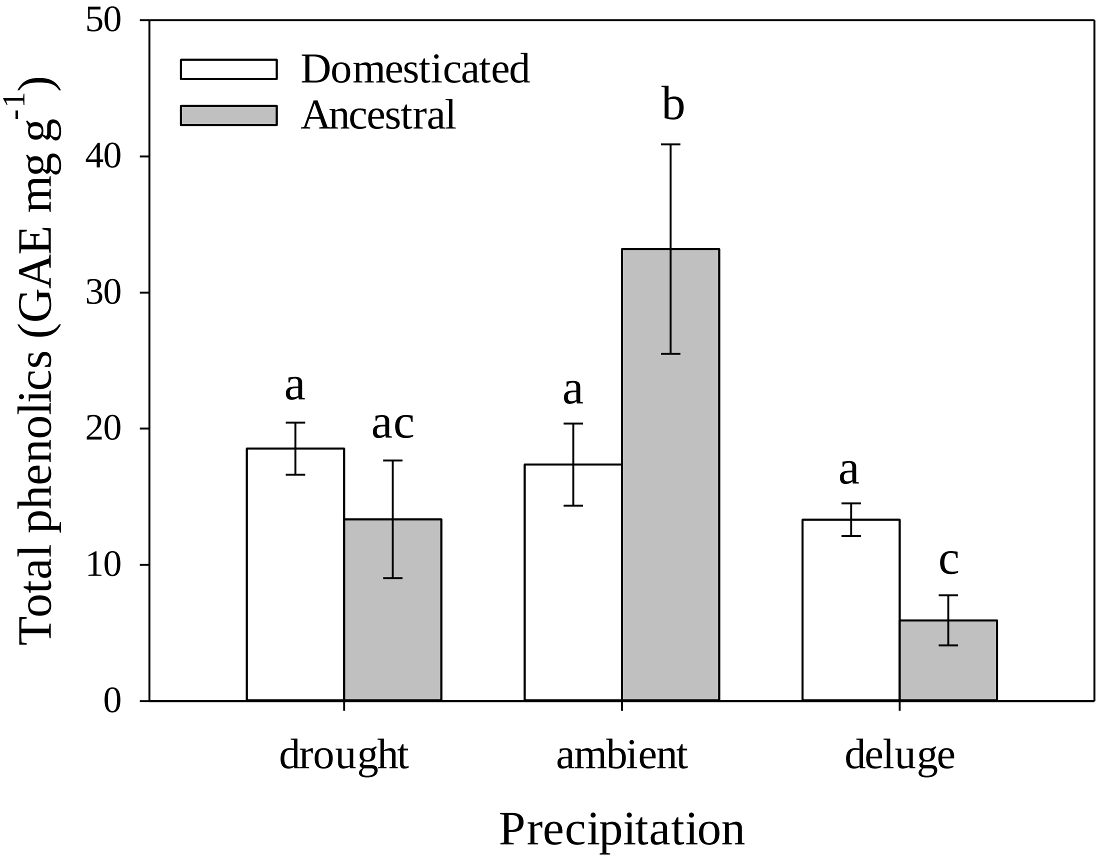

Fig. S3 The interactive effects of precipitation and domestication on total root phenolic concentrations. Mean values (±SE) are shown. Bars with the same letters were not significantly different ($P < 0.05$). GAE refers to Gallic Acid Equivalent.