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Plant functional groups mediate drought resistance and recovery in a multi-site grassland experiment

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Running Headline: Drivers of grassland drought responses

Abstract

1. Climate change predictions suggest that summer droughts will become more intense and recurrent in Europe. While drought-induced reductions in grassland primary productivity are well documented, the drivers behind drought resistance (the capacity to withstand change) and recovery (the capacity for recovery of function) of above- and belowground biomass remain poorly understood.

2. Across eight grasslands differing in plant community productivity (CP) we investigated the effects of summer drought on plant and soil microbial variables, plant nutrient content and soil nitrogen (N) availability. We examined the linkages between community productivity, soil N, drought responses of plant and microbial communities and relative drought responses of plant and microbial biomass. Plant and microbial variables were recorded at the end of a three-month rainfall exclusion period. Plant variables were also assessed during a 10-month drought recovery period.

3. Experimental drought decreased plant biomass and increased plant C:N ratios, but had no effect on total microbial biomass across sites. Instead, drought caused shifts in plant and microbial community structures as well as an increase in arbuscular mycorrhiza fungi biomass. Overall, plant biomass drought resistance was unrelated to community productivity or microbial community structure but was positively related to drought resistance of forbs.

4. In the month after rewetting, soil N availability increased in droughted plots across sites. Two months post-rewetting, droughted plots had higher plant N concentration, but lower plant N use efficiency. The short-term drought recovery of plant biomass was unrelated to community productivity or soil N availability, but positively related to the response of grass biomass, reflecting incomplete recovery at high community productivity. Ten months after rewetting, drought effects on plant biomass and plant N content were no longer apparent.

5. *Synthesis.* Our results suggest that drought resistance and recovery are more sensitive to plant community composition than to community productivity (CP). Short-term recovery of plant biomass may also benefit from increased soil N availability after drought and from a high abundance of soil fungi in low productivity sites. Our findings underline the importance of plant functional groups for the stability of permanent grasslands in a changing climate with more frequent drought.

Keywords

Fungi, land use intensity, NLFA, nitrogen, PLFA, precipitation manipulation, semi-natural grasslands, soil microbial community

Zusammenfassung

Funktionelle Pflanzengruppen bestimmen Trockenresistenz und Funktionsfähigkeit nach Dürre in einem Graslandexperiment an mehreren Standorten

1. Klimamodelle legen nahe, dass Sommertrockenheit in Europa intensiver und häufiger eintreten wird. Die durch Trockenheit bedingte Verringerung der primären Produktivität von Grasland ist gut dokumentiert. Die ökologischen Prozesse, welche die Trockenresistenz (die Fähigkeit, Veränderungen zu widerstehen) und die Wiederherstellung der ober- und unterirdischen Biomasse (die Fähigkeit zur Wiedergewinnung der Funktion) bestimmen, sind hingegen kaum untersucht.

2. In acht Wiesen, die sich in der Produktivität der Pflanzengemeinschaften unterscheiden, untersuchten wir die Auswirkungen von Sommertrockenheit auf die Pflanzen und Bodenmikroben, den Nährstoffgehalt der Pflanzen und die Verfügbarkeit von Stickstoff (N) im Boden. Wir untersuchten die Zusammenhänge zwischen Produktivität, N im Boden, Dürrereaktionen von Pflanzen- und Bodenmikroben-Gemeinschaften und relativen Dürrereaktionen der pflanzlichen und

mikrobiellen Biomasse. Pflanzen und Mikroben wurden am Ende eines dreimonatigen Zeitraums erfasst, der durch Regendächer niederschlagsfrei gehaltenen wurde. Zusätzlich wurden Pflanzenvariablen während 10 Monaten nach der Dürre erfasst.

3. Die experimentelle Dürre verringerte die Pflanzenbiomasse und erhöhte die C-N-Verhältnisse der Pflanzen, hatte aber keine Auswirkung auf die mikrobielle Gesamtbiomasse der untersuchten Wiesen. Die Dürre verursachte jedoch Verschiebungen in der Zusammensetzung der Pflanzen- und Mikroben-Gemeinschaften sowie eine Zunahme der Biomasse der arbuskulären Mykorrhizapilze. Insgesamt wurde die Trockenresistenz der Pflanzenbiomasse weder von der Produktivität noch von der Struktur der Mikroben-Gemeinschaft bestimmt. Die Trockenresistenz der Pflanzenbiomasse stand jedoch in einem positiven Zusammenhang mit der Trockenresistenz der Kräuter.

4. Im ersten Monat nach dem Wiederbefeuchten war die Verfügbarkeit von N im Boden in den zuvor niederschlagsfrei gehaltenen Parzellen erhöht. Zwei Monate nach dem Wiederbefeuchten zeigten Pflanzen in zuvor niederschlagsfrei gehaltenen Parzellen eine höhere N-Konzentration, jedoch eine geringere N-Nutzungseffizienz. Die rasch wiedergewonnene pflanzliche Biomasse stand in keinem Zusammenhang mit der Produktivität der Pflanzengemeinschaft oder der Verfügbarkeit von N im Boden, stand jedoch in einem positiven Zusammenhang mit der Reaktion der Biomasse der Gräser und widerspiegelte deren noch nicht vollständig wiedergewonnene Funktionsfähigkeit in Pflanzengemeinschaften mit hoher Produktivität. Zehn Monate nach dem Wiederbefeuchten waren keine Auswirkungen der Trockenheit auf die pflanzliche Biomasse und den N-Gehalt der Pflanzen mehr zu erkennen.

5. Synthese. Unsere Ergebnisse deuten darauf hin, dass Trockenresistenz und Funktionsfähigkeit nach Dürreperioden empfindlicher auf die Zusammensetzung als auf die Produktivität der Pflanzengemeinschaft reagieren. Die rasche Wiedergewinnung der Funktionsfähigkeit kann auch durch eine nach Dürreperioden erhöhte Stickstoffverfügbarkeit im Boden, und an Standorten geringer Produktivität, durch eine Fülle von Bodenpilzen begünstigt werden. Unsere Ergebnisse

unterstreichen die Bedeutung funktioneller Pflanzengruppen für die Stabilität des Dauergraslandes in einem sich verändernden Klima mit häufiger eintretenden Dürren.

1. Introduction

Climate change predictions suggest that central Europe will experience longer and more intense summer droughts in the future accompanied by an increase in summer temperatures (IPCC, 2013). Drought is of particular concern for permanent grasslands, which represent approximately 38% of agricultural land area in Europe (FAO, 2015), and can show high sensitivity to rainfall patterns (Sala, Parton, Joyce, & Lauenroth, 1988; Lane, Coffin, & Lauenroth, 2000; Ciais et al., 2005; Knapp et al., 2015). Recent work suggests that adjusting grassland management intensity, such as reducing mowing frequency, has the potential to improve grassland drought resistance and maintain yields (Vogel, Scherer-Lorenzen, & Weigelt, 2012), but the drivers of drought responses under different management systems remain unclear. Improved understanding of the importance of plant- and soil-based mechanisms underlying impacts of drought on grassland function is therefore critical for the development of effective adaptation strategies to climate change.

Drought-induced decreases in soil moisture have direct and indirect effects on plant productivity (Frank et al., 2015). In general, grassland productivity decreases during drought, although grasslands may show varying degrees of drought resistance, i.e., the capacity to withstand change (Kahmen, Perner, & Buchmann, 2005; de Boeck et al., 2006; Hoover, Knapp, & Smith, 2014). Variation in the drought resistance of biomass production across grasslands may partly be linked to differences in plant stress tolerance (Grime et al., 2008; Volaire, Barkaoui, & Norton, 2014) and/or plant productivity, since high biomass systems with high water demand are expected to decrease soil moisture and hence increase ecosystem vulnerability to drought (Wang, Yu, & Wang, 2007). Drought-induced reductions in plant biomass production during drought can promote soil nutrient

availability due to reduced uptake by plants (Homyak et al., 2016). Drought-induced decreases in soil moisture content may also modify soil microbial activity and/or community composition with consequences for substrate diffusion, soil nutrient retention and availability which feeds back to plant productivity (Schimel, Balser, & Wallenstein, 2007; Bloor & Bardgett, 2012; Frank et al., 2015). Water stress typically reduces microbial activities and substrate-use, with stronger negative effects on fast-growing bacteria compared to fungi (Manzoni, Schimel, & Porporato, 2012). Recent work suggests that microbial community composition, in particular increased abundance of slow-growing K-strategists, may increase the drought resistance of the microbial community (De Vries & Shade, 2013). Moreover, results from mesocosm experiments suggest that microbial biomass drought resistance may be negatively correlated to plant biomass resistance (Orwin & Wardle, 2005; Bloor, Zwicke, & Picon-Cochard, 2018), a phenomenon thought to be driven by associated changes in rhizodeposition. To date though, information on the effects of drought on coupled plant/microbial responses under field conditions is lacking (Mariotte, Robroek, Jassey, & Buttler, 2015; Karlowsky et al., 2017).

Despite variation in the level of drought resistance recorded for grassland biomass production, numerous studies suggest that the capacity of grasslands to recover function after drought is high (Pimm, 1984; Hoover et al., 2014). Plant biomass recovery after drought is generally fast, regaining ambient levels of production one year after the drought (Mariotte, Vandenberghe, Kardol, Hagedom, & Buttler, 2013; Hoover et al., 2014; Yang et al., 2016; Stampfli, Bloor, Fischer, & Zeiter, 2018). High abundance of grass species, with a capacity to pre-empt nitrogen and space by rapid re-sprouting from basal meristems may also contribute to fast biomass recovery in grassland systems (Volaire et al., 2014; Stampfli et al., 2018). However, drivers of grassland drought recovery remain unclear. Soil nutrient availability could play an important role in mediating plant recovery following stress, allowing re-growth of fast-growing plants when soil water availability improves (MacGillivray et al., 1995). This is of particular interest in the context of land use intensification, which is known to modify plant and soil properties via the application of fertilizers, an increase in

annual mowing frequency or the addition of grazing animals (Berner et al., 2011; Lavorel et al., 2011a). Intensively-managed grasslands are characterized by high productivity, fast-growing, resource-acquisitive plant species and bacterial-based food webs (Lavorel et al., 2011b; De Vries et al., 2012; Grigulis et al., 2013). In contrast, extensively-managed grasslands are dominated by slow-growing, resource-conservative and stress-tolerant plant species with low productivity, which promote fungal and K-strategist-based food webs with slow nutrient cycles and low soil N availability (De Vries et al., 2012; Grigulis et al., 2013). In theory, the fast-growing plant species and inherently higher nutrient availability in productive grasslands should promote grassland recovery after drought. Plant recovery in productive grasslands could be further enhanced by shifts in competition for resources between plants and microbes due to the low drought resistance of the bacteria-dominated microbial community in these systems (Borken & Matzner, 2009).

Here we use an *in situ* drought experiment across eight sites in Switzerland to examine the drought responses of hay meadows with differing levels of plant community productivity, due to past management practices. We investigated plant and soil microbial community responses to an extended summer drought and monitored drought recovery of the plant community for 10 months post-rewetting. The primary objective of this study was to examine the linkages between plant community productivity, resource availability, plant and microbial community composition and the stability of plant and microbial biomass under drought. We expected that summer drought would decrease both plant and soil microbial biomass, and hypothesized that: (H1) highly productive sites have a lower microbial resistance to drought than low productivity sites due to a soil microbial community with a lower relative abundance of K-strategists (De Vries & Shade, 2013); (H2) highly productive sites have a higher plant recovery to drought than low productivity sites due to greater resource availability and a higher abundance of resource-acquisitive plant and microbial species, which increase plant production recovery (Grigulis et al., 2013). The overarching objective of this multi-site drought experiment is to provide insights into the biological drivers of drought resistance and recovery to support semi-natural grassland management.

2. Materials & Methods

2.1 Study site and field experiment

Eight permanent grassland sites were selected at upland elevations (555 – 1110 m a.s.l.) across the Central Plateau and the Northern, Central and Southern Alps of Switzerland in March of 2014. All sites have been under continuous grassland without ploughing for at least three decades, but sites vary in terms of soil properties and land use intensity, which has modified plant community structure and functioning over time (Table 1). In our study, intensively-managed sites (cut more than twice per year in the past) had greater community productivity (CP) and community-weighted means of specific leaf area (SLA) than extensively-managed sites (cut once or twice per year in the past), based on aboveground harvests and species frequency measures taken in 2014 before the start of experimental treatments (Table 1, Table S.1, Appendix S.1). Our intensively managed sites also had greater annual net primary productivity (ANPP) based on harvests taken from the ambient treatment in 2014 (Table S.1). Plant species richness was not related to community productivity across sites (r = -0.30, $P > 0.05$, n = 8). In general, grasses had a significantly higher community-weighted mean of SLA than forbs across our study sites ($t_7 = -2.44$, $P < 0.05$, Table S.1) and are therefore considered more 'resource acquisitive' according to Reich (2014).

The experiment was designed with two treatments: total precipitation exclusion from mid-June until the end of August 2014 (DRY) and ambient precipitation (AMB). Precipitation was manipulated using rainout shelters constructed with 20°-inclined roofs made of 90% UV-transparent plastic material (3.8 × 4.5 m) and fixed to a wooden frame. During experimental manipulation, rainout shelters were applied to two of four plots (2 × 2 m) positioned randomly per site. The shelters covered the plots at a height of 80 cm and reduced photosynthetically active radiation by ca. -11%. At the end of the drought period, DRY plots were re-wetted with at least 25 l H_2O m^{-2}, either from natural precipitation at the site or through manual water additions during the 10-day re-wetting period (Table S.2). After this time, all plots received natural rainfall until the end of the

experiment.

Soil moisture content during drought was monitored using one soil moisture sensor (TMS3, TOMST® Measuring System, Czech Republic) placed in each plot to a depth of 10 cm from the beginning of May 2014 until the end of October 2014. A sensor measuring dielectric soil water potential (MPS-1, Decagon Devices, USA) was used to convert daily means of the soil moisture sensors into soil water potential values (Stampfli et al., 2018). Dry spell length per site and treatment (Table 2) was calculated by summing the number of consecutive days when soil water potential was $\psi < -100$ kPa from the beginning of summer until post-drought rewetting. Desiccation below $\psi = -100$ kPa, known as the "refill point" in agricultural science, impairs plant growth (Merot, Wery, Isbérie, & Charron, 2008; Shock & Wang, 2011). Soil moisture for deeper soil layers was determined gravimetrically and samples were extracted using an Edelman auger with a diameter of 6 cm (Table 2).

2.2 Plant sampling & analyses

Aboveground plant biomass samples were taken from two 30 × 60 cm subplots per plot at four sampling dates: mid-June (beginning of drought), end of August (end of drought) and end of October 2014 (end of growing season, two months post-rewetting), as well as mid-June 2015 (peak biomass, 10 months post-rewetting). All living aboveground plant biomass was cut to a height of 4 cm above the soil. The samples were sorted into functional groups, grasses (including all graminoids) and forbs (including non-gramineous herbs and woody dwarf shrubs), and forbs were further separated into leguminous and non-leguminous forbs by hand. All biomass samples were dried (60°C for 48 hours) prior to weighing. After weighing, dried biomass samples were pooled per subplot and homogenized using a cutting mill (1 mm mesh size, Retsch, WRb90), and a 3 g subsample of each mixture was then finely ground (Brinkmann ball grinder, Retsch, MM200). Total C and N content in aboveground biomass samples were determined for 5 mg of finely ground material (Brinkmann ball

grinder, Retsch, MM200) using an elemental combustion analyzer (Flash EA 1112 CNS analyzer, ThermoFinnigan, Milan, Italy). Data on shoot C and N were used to determine plant C:N ratios and aboveground N stocks (N_{plant}). Nitrogen use efficiency (NUE) was assessed using the biomass:N content ratio (Fargione & Tilman, 2006).

2.3 Soil sampling & analyses

Soil samples were taken in August 2014, at the end of drought and immediately prior to re-wetting. Soil samples (surface of 144 cm^2) were taken to a depth of 10 cm from each of four 50 × 40 cm subplots located at the center of each plot. Soil samples were pooled per plot and sieved (2 mm mesh) to homogenize the soil of all four soil subplots. A subsample was stored in a -80°C freezer until further analyses. All instruments used for soil sampling and sieving were surface-sterilized in 70% Ethanol to prevent cross-contamination between samples.

Two grams of soil were taken for lipid extraction and fractionation following the alkaline methylation method (Frostegård, Tunlid, & Bååth, 1991). The resulting phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) methyl ethers (MEs) were dissolved in isooctane and measured by gas chromatograph using an Auto System XL (PerkinElmer, USA) using an HP-5 capillary column, a flame ionization detector and helium as the carrier gas. Fatty acid methyl esthers (FAMEs) were identified using their retention time based on fatty- and bacterial-acid methylether-mix (Sigma-Aldrich, USA). Quantification of PLFA and NLFAs was calculated with the use of an internal FAME standard, which had been added before methanolysis. Nomenclature and division of PLFAs into bacteria and fungi was based on Kandeler et al. (2008), Frostegård and Bååth (1996) and Zelles (1999). Gram-positive bacteria (Gram+) were represented by PLFA biomarkers i15:0, a15:0, i16:0, and i17:0. Gram-negative bacteria (Gram–) were represented by PLFA biomarkers cy17:0 and cy19:0. Total bacteria (Bacteria) were represented by the sum of PLFA biomarkers for Gram+, Gram– and 16:1ω7, a generalist bacterial biomarker. Saprotrophic fungi (SF) was represented by 18:2ω6. The

saprotrophic fungi:bacteria ratio was used as an indicator of the abundance of K-strategists (De Vries & Shade, 2013). Total PLFAs were used to represent microbial biomass and included Gram+, Gram–, SF as well as the 'nonspecific bacteria and fungi' biomarkers 16:1ω7 and 16:1ω5, which represent generalist bacteria and a mix of arbuscular mycorrhiza fungi and Gram–bacteria, respectively. Relative abundances of bacterial and fungal groups (Gram+, Gram–, SF and 'nonspecific') were calculated relative to total microbial biomass. The NLFA biomarker 16:1ω5, which represents patterns based on storage lipids, was used to represent arbuscular mycorrhiza fungi (AMF) following Fuchslueger, Bahn, Fritz, Hasibeder and Richter (2014) and Karlowsky et al. (2017). This NLFA biomarker is considered to be more reliable than the PLFA biomarker 16:1ω5 for soils that have a high abundance of bacteria (Joergensen & Wichern, 2008; Frostegård, Tunlid, & Bååth, 2011).

In September 2014, and again at the beginning of October 2014, 'plant root simulator' probes (PRS^{TM}-probes, Western AG, Canada) were inserted into each plot at a depth of 3 to 8.5 cm to determine soil mineral N availability. PRS-probes were left in place for four and eight weeks during the two measurement periods, respectively, then extracted and washed in deionized water prior to analysis by the manufacturer. Total soil inorganic nitrogen (N_{soil}) was obtained from two pooled samples each composed of four cation and four anion probes per plot. The site ZOL was removed from the statistical analysis of these variables due to unreasonably high values in DRY plots, likely due to the presence of feline feces (observed when collecting the probes).

2.4 Statistics

Effects of drought, community productivity (CP) and their interaction on absolute values of plant, microbial and abiotic variables were evaluated using two-way linear models. Drought was a categorical factor (AMB, DRY) and nested within site, whereas CP was a continuous predictor based on site productivity recorded prior to drought treatment in 2014 (Table 1). Where data was sampled on multiple dates, each date was analyzed separately. We applied ANCOVA to biomass variables, soil

moistures and N contents of soil; ANCOVA was applied as we only have two blocks per site, which is below the minimum levels recommended for random effects in a mixed model (Bolker et al., 2018). Data was log-transformed when homogeneity of variance was not achieved with raw values. We applied generalized linear model (GLM), with binomial distribution and logit-link function, to proportions data (e.g., relative abundances of functional groups). We produced accumulated analyses of deviance tables and tested the effect of CP against site, all other factors were tested against the residual using quasi-F tests (McCullagh & Nelder, 1989).

Where drought effects were significant, response ratios ($RR_{DRY / AMB}$) were calculated from site means. A RR value of one indicates no difference between DRY and AMB plots, while values below one indicates lower values in DRY plots. We tested whether RR, i.e., relative drought effects, were related to CP using regression analysis. Regression analysis was also used to test whether biomass resistance to drought (i.e., the relative drought effects at the end of drought) could be explained by changes in plant or microbial community composition. Finally, we used regression analysis to examine if plant biomass recovery (post-rewetting RR) could be explained by changes in plant community composition or nutrient availability in soil. The R statistical program was used for all statistical analyses (version 3.2.2., R Core Team, 2015).

3. Results

3.1 Soil moisture during drought

Dry spell length, i.e., the number of consecutive days when $\psi < -100$ kPa, was higher for DRY plots than AMB plots (78 versus 11 days on average respectively, $F_{1,6} = 240.8$, $P < 0.001$; Table 2, Fig. S.1). Gravimetric topsoil moisture was lower in DRY plots at the end of drought (-67.5% on average,

$F_{1,15}$ = 22.8, P < 0.001). Soil moisture content in the deeper soil layers of 25 – 40 cm and 45 – 60 cm was also lower in DRY plots ($F_{1,13}$ = 6.9, P < 0.05 and $F_{1,11}$ = 5.2, P < 0.05, respectively; Table 2). Community productivity (CP) had no significant effect on soil moisture.

3.2 Plant and microbial responses to drought

At the end of experimental drought, total plant biomass was lower in DRY compared to AMB plots across sites (-79% on average across sites, Fig. 1A). Total plant biomass showed a significant interaction between drought and CP; the magnitude of drought-induced decreases was greater with increasing CP (Fig. 1A). However, biomass drought response ratios (RR) were not related to CP across sites (P > 0.05). Drought caused reductions in the biomass of all plant functional groups (inset Fig. 2A, Table S.3), but only grass biomass mirrored the interaction between drought and CP observed for total plant biomass (Fig. 1C).

Drought had a relatively larger effect on grasses than on forbs (inset Fig. 2A). Moreover, the negative drought effect was larger for leguminous than for non-leguminous forbs (Fig. S.2A). Consequently, drought reduced the relative abundance of grasses, but increased the relative abundance of forbs (Table S.4). Total plant biomass resistance (RR at the end of drought) was positively related to the resistance of forbs (RR at the end of drought) (Fig. 2A), but was unrelated to RR in grasses.

Aboveground plant C:N was higher at the end of drought in DRY compared to AMB plots (+24% on average, respectively Fig. 3A), whereas plant N concentration (N_{plant}) was lower in DRY plots across sites (-12% on average, Fig. 3B). NUE was not affected by drought (Fig. 3C). Drought-induced changes in N_{plant}, plant C:N and NUE were all of smaller magnitude with increasing grassland productivity (significant CP × drought interactions, Fig. 3).

Total microbial biomass showed no significant response to either drought treatment or CP at the end of experimental drought (Table 3). However, different microbial groups varied in their response to drought. Absolute biomass of PLFA Gram+, Gram– bacteria and SF showed no response to drought, whereas NLFA AMF increased (+66% on average) and PLFA 'nonspecific bacteria and fungi' decreased (-18% on average) in DRY plots at the end of drought (Fig. 4). In addition, both Gram+ and Gram– bacteria groups and the 'nonspecific' group increased in biomass with increasing CP (Table S.5).

Drought had a positive effect on the relative abundances of Gram+ bacteria and saprotrophic fungi, but a negative effect on the relative abundance of 'nonspecific bacteria and fungi' (Table 3). The relative abundance of saprotrophic fungi and the fungi:bacteria ratio showed interactions between drought and CP (Fig. S.3A, S.4). In sites with low productivity, DRY plots had a greater proportion of fungi in the soil, whereas no difference was observed between DRY and AMB plots under highly productive sites. Drought-induced decreases in relative abundance of 'nonspecific microorganisms' increased in magnitude with increasing CP (Fig. S.3B). Drought-induced changes in microbial community composition were not related to total plant biomass resistance (data not shown).

3.3 Soil nitrogen availability after drought

In the month directly following the end of drought, soil mineral N availability (N_{soil}) was higher in DRY plots compared to AMB plots (+219% on average, Fig. 5A). During this period, drought-induced increases in N_{soil} were greater at sites with increasing CP (Fig. 5A). Drought-induced changes in N_{soil} were no longer apparent after the first month, while N_{soil} still showed a positive relationship with CP two to three months post-rewetting (Fig. 5B).

3.4 Plant recovery after drought

Drought-induced reductions in total plant biomass were still apparent two months post-rewetting, in particular for plots with high productivity (Fig. 1B). This response pattern was also observed for grass biomass (Fig. 1D), although the overall effect of drought on grasses (pooling values at all levels of CP) was no longer significant at this time (inset Fig. 2B). Short-term drought recovery of total plant biomass (RR two-months post-rewetting) was positively related to the recovery of grass biomass (RR two months post-rewetting) (Fig. 2B). Grass biomass recovery was also negatively related to grass community-weighted mean SLA (R^2 = 0.82, P < 0.001, data not shown). Drought RR of plant biomass did not show any relationship with CP or soil N availability.

Two months after rewetting, drought had a negative effect on the absolute abundance of forbs (inset Fig. 2B, Table S.3), driven by persistent drought effects on leguminous forbs (Fig. S.2B, Table S.3). Nevertheless, drought-induced increases in the relative abundance of forbs were no longer apparent (Table S.4). Overall, ANPP in 2014 was lower in DRY plots compared to AMB plots (556.0 g m^{-2} and 729.4 g m^{-2}, respectively; $F_{1,7}$ = 225.2, P < 0.001, Table S.1). Drought-induced reductions in ANPP were higher with increasing grassland productivity (DROUGHT × CP interaction, $F_{1,21}$ = 50.6, P < 0.001).

Two months post-rewetting, drought continued to effect plant nutrient content and nutrient use (Fig. 6). Irrespective of CP, N_{plant} was higher (+13% on average), while plant C:N and NUE were lower in DRY compared to AMB plots (mean decrease of -9% and -11%, respectively; Table S.6).

Ten months after rewetting, drought effects were no longer detected for plant biomass (total, grass and forb), N_{plant}, plant C:N or NUE (Table S.3, S.6). Only leguminous forb biomass continued to display a negative effect of drought in the previously-droughted plots (-53% on average across sites; Fig. S.2C). As before, total plant and grass biomass showed positive relationships with CP across all plots ($F_{1,7}$ = 56.6, P < 0.001; $F_{1,7}$ = 229.9, P < 0.001, respectively).

4. Discussion

Broad-scale experiments which include measurements of plant and microbial responses are essential for the appraisal and forecasting of ecosystem vulnerability to precipitation extremes in terrestrial systems (Beier et al. 2012). Our simulation of severe summer drought across eight permanent grasslands demonstrated differences in the drought responses of aboveground plant biomass and soil microbial biomass. Rainfall exclusion for a duration which has not recurred in 50 years (except for sites in the Central Alps, Stampfli et al., 2018), caused strong decreases in plant biomass at the end of drought, in line with previous field drought experiments (Kahmen et al., 2005; Hoover et al., 2014; Stampfli et al., 2018). Severe drought also decreased forage quality (increased plant C:N ratio), consistent with drought-induced changes in plant physiology and leaf senescence (van der Molen et al., 2011). Contrary to expectations, total microbial biomass showed no response to drought, due to mixed drought responses in microbial groups, while AMF increased under drought as observed in other studies (Orwin & Wardle, 2005; Karlowsky et al., 2017).

Although drought-induced reductions in soil moisture generally decrease soil microbial activities via a combination of direct and indirect effects (Manzoni et al., 2012) field studies have reported mixed responses of microbial biomass to drought (Gordon, Haygarth, & Bardgett, 2008; Sheik et al., 2011; Fuchslueger et al., 2014). Drought resistance of microbial biomass can be linked to soil and vegetation properties, as well as to microbial community structure and dormancy strategies in microorganisms (Griffiths & Philippot, 2012; Shade et al., 2012). Seasonal variation in microbial activity may also contribute to variation in drought responses, since microorganisms are less vulnerable to environmental fluctuations during naturally inactive periods (Lauber, Ramirez, Aanderud, Lennon, & Fierer, 2013). In the present study, impacts of drought on microbial biomass may have been buffered by shifts in microbial community structure. The relative increase of Gram+ bacteria and saprotrophic fungi suggests a change within the microbial community to resist drought stress, likely due to their more tolerant morphology such as Gram+ bacteria's thick peptidoglycan

layer (Schimel et al., 2007; Evans & Wallenstein, 2014). Microbial biomass stability may also have been mediated by shifts in assimilate allocation in the plant-soil system (Sanaullah, Chabbi, Rumpel, & Kuzyakov, 2012; Karlowsky et al., 2017). Drought-induced changes in the source-sink relationship of plants can modify the supply of labile substrates to microorganisms, with consequences for rhizomicrobial activity and the osmotic adjustment of soil microorganisms (Karlowsky et al., 2017). A decrease in nutrient exchange or competition for nutrients between plants and soil microorganisms can result in their decoupling and has implications for overall system functioning, such as changes to substrate pools, and a divergence in their responses to drought (Bloor & Bardgett, 2012; Fuchslueger et al., 2014; Karlowsky et al., 2017).

Given that highly productive grasslands are characterized by a low fungi:bacteria biomass ratio (Grigulis et al., 2013), and that fast-growing, r-strategist microorganisms are considered to be less resistant to environmental fluctuations than slow-growing fungi or K-strategists (De Vries & Shade, 2013), we predicted that highly productive sites would have a lower total microbial resistance to drought. Our findings did not support this hypothesis. Although the abundance of bacterial groups increased with increasing community productivity across our study sites and fungi abundance was within the range of other grassland studies (Bardgett & McAlister, 1999; Karlowsky et al., 2017; Pommier et al., 2017), microbial drought resistance was unrelated to community productivity. In addition, neither community productivity nor microbial community structure showed any relationship with the drought resistance of plant biomass, suggesting that these two biotic factors play a limited role for variation in plant drought resistance. Instead, we found that drought resistance of forbs promoted total plant biomass resistance. This confirms the importance of plant functional groups for grassland responses to drought (Fry et al., 2013; Stampfli et al., 2018).

Despite the low drought resistance in plant biomass observed across all sites, drought recovery was high; all drought legacy effects on plant biomass had disappeared within ten months, in agreement with fast grassland biomass recovery reported elsewhere (Mariotte et al., 2013;

Hoover et al., 2014; Yang et al., 2016; Stampfli et al., 2018). Drought recovery was almost certainly promoted by a drought-induced increase in soil N availability since all droughted plots showed higher mineral N supply rates in the month after rewetting (Fig. 5A), and increased plant nitrogen content two months after rewetting (Fig. 6B). Increases in soil N availability are consistent with reduced plant N uptake during drought, and an increase in microbial activity and a pulse in soil C and N mineralization following rewetting (Birch, 1958; Fierer & Schimel, 2002; Borken & Matzner 2009; Homyak et. al., 2016). Increase in plant-available N, coupled with upregulation in photosynthetic activities after drought, drive short-term increases in forage quality after rewetting (Bloor & Bardgett, 2012; Niboyet, Bardoux, Barot, & Bloor, 2017). However, we did not find a clear-cut relationship between soil mineral N availability (post-rewetting) and the short-term drought recovery of plant biomass (assessed two months after rewetting). This lack of relationship between soil N availability and plant recovery rates suggests that even the smallest drought-induced increases in soil N may have been sufficient to support plant recovery at our sites (as seen with increased plant N content in droughted plots across sites). Variation in drought-induced increases in soil N may partly have been buffered by plant N use efficiency across sites, since we found that sites with lower soil N at the end of drought displayed higher plant NUE.

In the present work, we predicted that higher abundance of resource acquisitive plant and microbial groups in highly productive sites would promote the short-term drought recovery of plant biomass. This hypothesis was not supported by our data, but instead our results confirm recent findings from a separate study of 12 grasslands in Switzerland (Stampfli et al., 2018). In their study, Stampfli and coworkers found that compensatory growth by grasses had a stabilizing effect on biomass production across sites of contrasting land use intensity. In the present study, we found some evidence of compensatory growth by grass species at low productivity (Fig. 1D). Moreover, high recovery of grasses at two months post-rewetting was associated with high plant biomass recovery at the same time point (Fig. 2B). Limited associations between community productivity and drought recovery in plant biomass may also reflect relatively more important plant-fungi

interactions in low productivity sites (Karlowsky et al., 2017). It is notable that the relative abundance of saprotrophic fungi and the fungi:bacteria ratio was higher in our dry, low productivity plots at the end of drought (Figs. S.3A, S.4). Fungi may promote drought recovery in plants, and resist decoupling (Fuchslueger et al., 2014), by extending the root network of plants and improving access to water and nutrients post-rewetting through hydraulic relocation, mycelia networks and hyphae of both saprotrophic and AM fungi (Wardle et al., 2004; Lau & Lennon, 2012; Guhr, Marzini, Borken, Poll, & Matzner, 2016). In addition, grasslands with a high abundance of fungi have been shown to maintain larger soil nutrient pools during drying-rewetting periods (Gordon et al., 2008; Martínez-García, De Deyn, Pugnaire, Kothamasi, & van der Heijden, 2017). Shifts in plant-soil feedbacks and/or competition for N, which increase plant N uptake in the presence of fungi, may further enhance plant recovery after drought (Kaisermann, de Vries, Griffiths, & Bardgett, 2017).

The low resistance and fast recovery of plant biomass observed across our study sites is broadly consistent with the idea that ecosystem resistance and recovery may be inversely related (De Keersmaecker et al., 2016; Karlowsky et al., 2017). It is notable that the more resource-conservative forbs determined biomass resistance and the more resource-acquisitive grasses determined biomass recovery, suggesting that plant resource-use strategies may play an important role in the tradeoff between drought resistance and recovery in grassland biomass. Overall, our findings indicate that community productivity may not be a reliable indicator of resistance or short-term recovery of grassland biomass to summer drought in cross-site comparisons. Instead, our results suggest that linkages between community productivity and drought recovery in plant biomass may be confounded by the abundance of plant functional groups. We propose that adjusting grassland management to support a conservative plant community composition may enhance the stability of biomass production in a future climate with longer and more intense summer droughts. Future studies should examine the role of soil microorganisms in plant biomass drought recovery and investigate the flow of soil nutrients above- and belowground post-rewetting under different land use intensities.

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Author's Contributions

MZ and AS conceived the ideas and designed methodology; MZ, AS, KAM and JB collected the data; KAM and MZ analyzed the data; KAM and JB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data Accessibility

Data available from the Dryad Digital Repository (Mackie, Zeiter, Bloor, & Stampfli, 2018).

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Figure Legends

Fig. 1. Effects of drought on total plant and grass biomass recorded at the end of experimental drought and two-months post-rewetting for ambient (AMB) and droughted (DRY) plots along a community productivity gradient (CP). Black and gray regression lines indicate AMB and DRY plots, respectively. F-values are presented for significant treatment effects based on two-way linear models. Data shown is of plots nested within eight sites.

Fig. 2. (A) Relationship between drought resistance in total plant biomass and forb biomass drought resistance. Inset: Absolute biomass of grass and forb plant functional groups in ambient (dark grey) and droughted (light grey) plots at the end of drought (means ± SE, n = 8 sites). (B) Relationship between drought recovery in total plant biomass and grass biomass drought recovery. Inset: Absolute biomass of grass and forb plant functional groups for ambient (dark grey) and droughted

(light grey) plots recorded two months post-rewetting (means ± SE, n = 8 sites). Stars represent significance, where $P < 0.05$ (*), $P < 0.001$ (***).

Fig. 3. Effects of drought on plant C:N, plant N and nitrogen use efficiency (NUE) (A,B, and C, respectively) recorded at the end of experimental drought for ambient (AMB) and droughted (DRY) plots along a community productivity gradient (CP). Black and gray regression lines indicate AMB and DRY plots, respectively. F-values are presented for significant treatment effects based on two-way linear models. Data shown is of plots nested within eight sites.

Fig. 4. Effects of drought on absolute biomass of microbial functional groups in ambient (AMB) and droughted (DRY) plots at the end of the experimental drought (means ± SE, n = 8 sites). Gram-positive (Gram+) and gram-negative (Gram–) bacteria, saprotrophic fungi (saprotroph) and 'nonspecific bacterial and fungal biomarkers' (nonspecific) were determined by phospholipid fatty acid (PLFA) biomarkers. Arbuscular mycorrhiza fungi (AMF) was determined by a neutral lipid fatty acid (NLFA) biomarker. Stars represent significance, where $P < 0.01$ (**).

Fig. 5. Effects of drought on soil mineral nitrogen availability (N_{soil}) recorded at (A) one month and (B) two to three months post-rewetting for ambient (AMB) and droughted (DRY) plots along a community productivity gradient. Black and gray regression lines indicate AMB and DRY plots, respectively. F-values are presented for significant treatment effects based on two-way linear models. Data shown is of plots nested within seven sites.

Fig. 6. Effects of drought on plant C:N, plant N concentration and nitrogen use efficiency (NUE) (A, B and C, respectively) two months post-rewetting for ambient (AMB) and droughted (DRY) plots (means ± SE, n = 8 sites). Stars represent significance, where $P < 0.01$ (**)

Tables

Table 1. Location and characteristics of the eight experimental grassland sites in Switzerland. Soil, land use and plant community properties are presented; abbreviations are given for bulk density (BD) from 0-4 cm soil depth, water holding capacity (WHC), fertilization since 1990 (Inputs), cutting frequency (Cuts, "+" indicates autumn grazing), low and high land use intensity (LUI), plant species richness (SR), community weighted mean of specific leaf area (SLA) and community productivity (CP) before drought in 2014.

Site	Code	Geographic Location (region)	Latitude, Longitude	Soil Type	Soil Texture	Skeleton (% vol.)	BD	WHC ($l m^{-2}$)	pH	Inputs	Cuts	LUI	SR (30 x 60 cm)	SLA ($m^2 kg^{-1}$)	CP ($g m^{-2}$)
Bister-Chumme	BCH	Cent. Alps	46.3643, 8.0750	regosol	loamy sand	<5	1.20	30	7.1	-	1	low	15.9	19.9	178
Thun	THU	N. Alps	46.7457, 7.5886	fluvisol	loamy sand	20-30	1.28	30	6.8	-	1	low	22.5	19.2	215
Negrentino	NEG	S. Alps	46.4621, 8.9241	acidic luvisol	sandy loam	<5	0.93	60	5.2	-	2	low	35.5	22.8	298
Krauchtal	KRA	Plateau	47.0096, 7.5705	regosol	loamy sand	<1	1.14	40	7	-	2	low	12.9	20.6	309
Somazzo	SOM	S. Prealps	45.8795,	cambisol	sandy loam	10-20	1.04	125	5.5	cow	2+	high	27.1	26.7	397

			8.9943							manure					
Zollikofen	ZOL	Plateau	46.9953, 7.4615	luvisol	loam	<5	1.27	125	5.4	-	3	high	15.6	27.3	545
Casserio	CAS	S. Alps	46.4417, 8.9355	leptosol	sandy loam	<5	1.02	40	5.4	slurry	3+	high	20.0	26.9	658
Bister-Breite	BBR	Cent. Alps	46.3614, 8.0617	phaeozem	loamy sand	<5	1.20	110	6.9	goat manure	2+	high	14.0	26.6	772

Table 2. Duration of edaphic drought stress and gravimetric soil moisture for ambient (AMB) and droughted (DRY) treatment (TRT) plots recorded at the end of experimental drought at the eight grassland sites (site codes given in Table 1). Values presented are the number of consecutive days of drought (CDD) where soil water potential at 0 – 10 cm soil depth ψ < -100 kPa and gravimetric soil moisture (%) at varying soil depths.

Site	TRT	CDD	Soil moisture (%)		
			5 – 20 cm	25 – 40 cm	45 – 60 cm
BCH	AMB	23	15.6	6.8	7.9
	DRY	80	2.7	2.5	.
THU	AMB	0	27.6	.	.
	DRY	61	3.7	.	.
NEG	AMB	0	39.1	32.1	32.5
	DRY	84	10.7	9.7	10.8
KRA	AMB	12	20.4	13.3	10.6
	DRY	79	3.7	2.9	2.7
SOM	AMB	0	32.9	28.8	27.1
	DRY	82	10.4	9.5	.
ZOL	AMB	0	27.1	20.0	16.8
	DRY	73	8.9	9.9	10.4
CAS	AMB	13	27.4	20.5	19.0
	DRY	78	21.1	20.3	14.8
BBR	AMB	36	16.7	14.5	13.6
	DRY	85	5.9	6.5	7.4

Table 3. Total microbial biomass and the relative abundance of microbial groups derived from PLFA biomarkers in ambient (AMB) and droughted (DRY) plots at the end of experimental drought. F-values of effects of community productivity (CP), drought (DROUGHT) and their interaction, P < 0.05 (*), P < 0.01 (**), P < 0.001 (***). Direction of relationship with CP is represented by (-).

	TRT	Mean	SE	CP $F_{1,7}$	DROUGHT $F_{1,21}$	CP×DROUGHT $F_{1,21}$
Absolute biomass (nmol PLFA g^{-1} dry matter)						
Total microbes	AMB	76.9	±6.3	ns	ns	ns
	DRY	72.2	±5.2			
Relative abundance						
Gram+ bacteria	AMB	0.50	±0.02	ns	10.5**	ns
	DRY	0.52	±0.02			
Gram– bacteria	AMB	0.08	±0.00	ns	ns	ns
	DRY	0.08	±0.00			
Saprotrophic fungi	AMB	0.09	±0.01	14.8** (-)	10.9**	7.9*
	DRY	0.11	±0.02			
Nonspecifc bacteria & fungi	AMB	0.33	±0.01	ns	59.3***	6.1*
	DRY	0.29	±0.01			

Figures

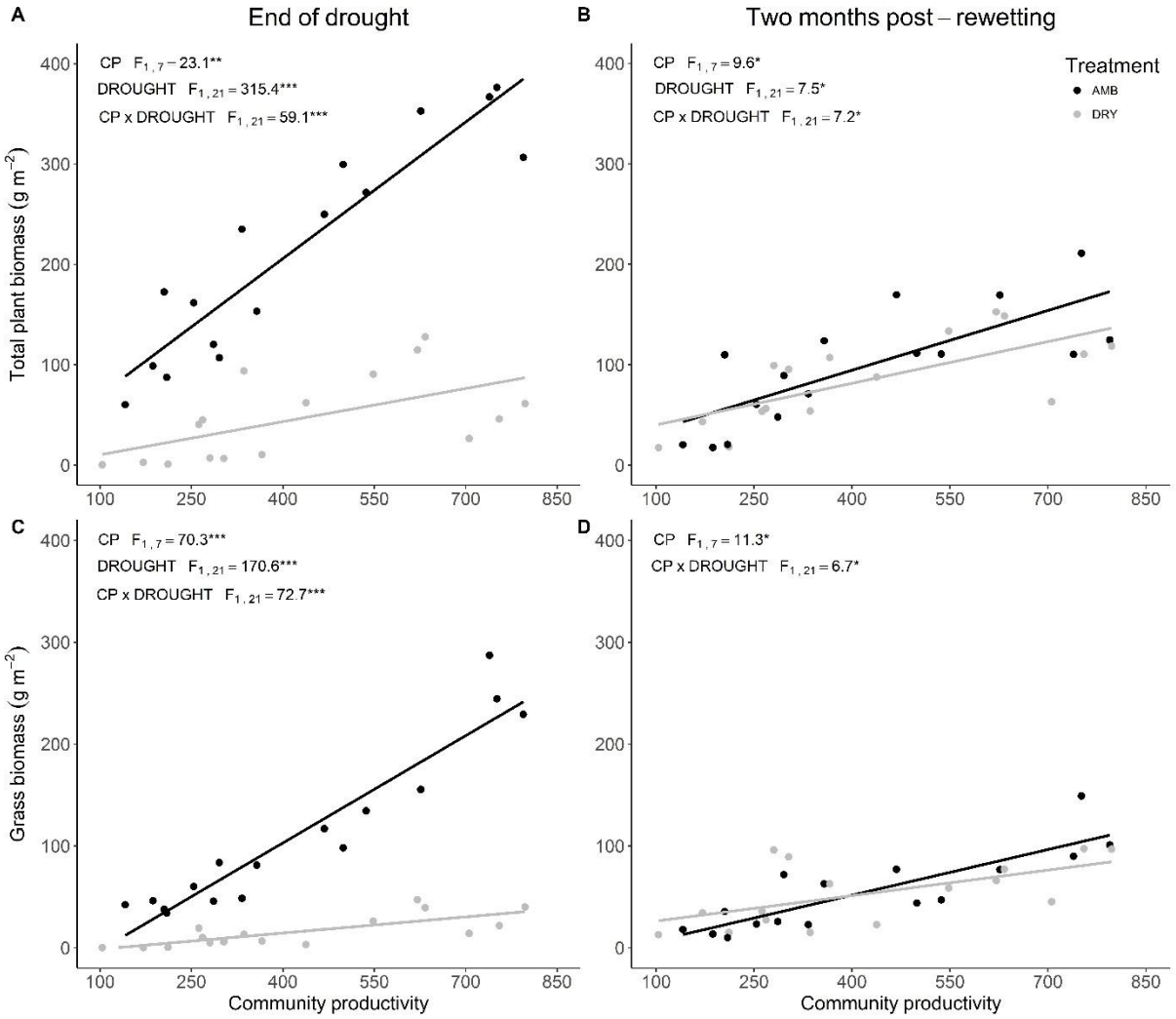

Fig. 1

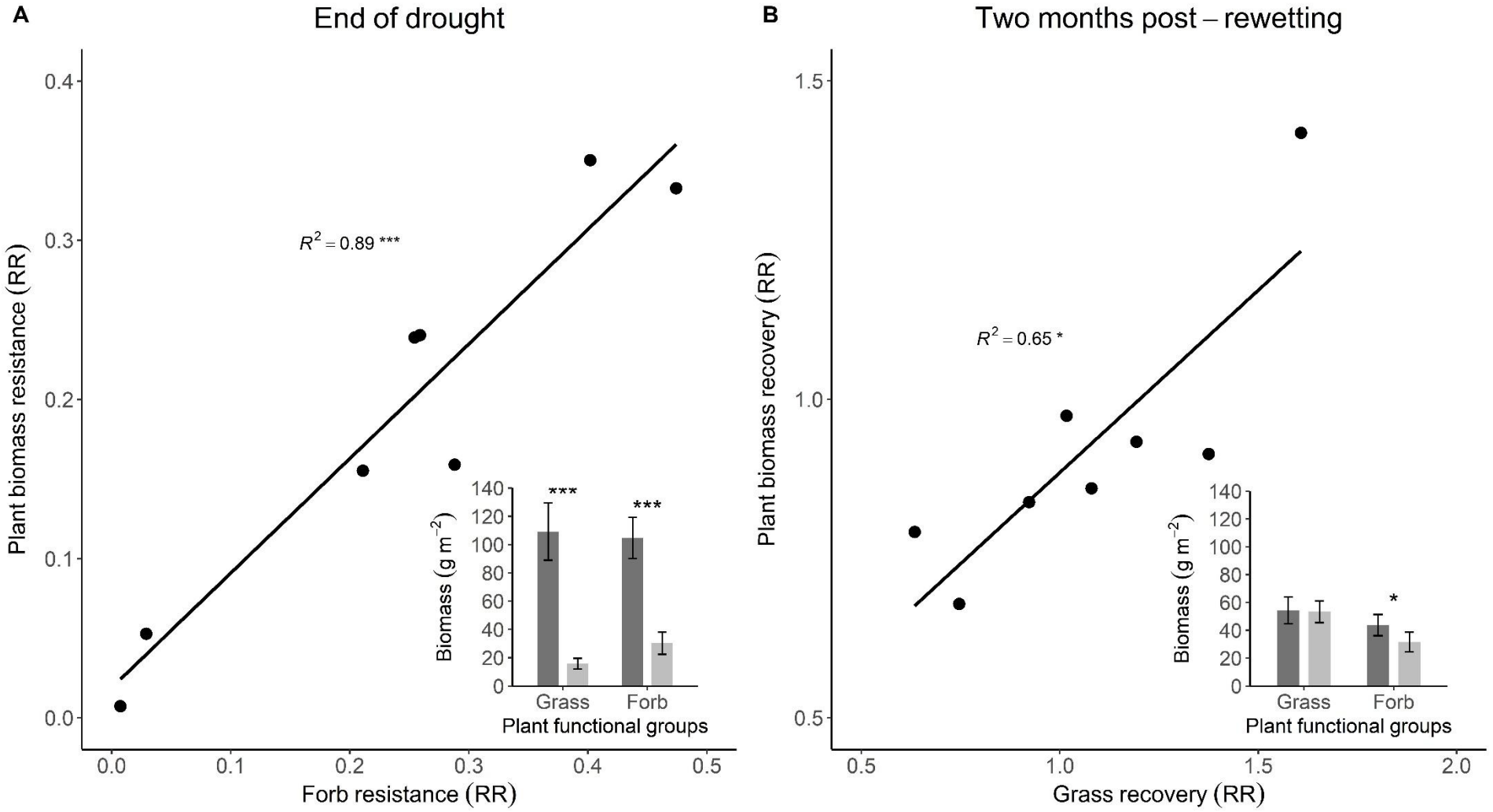

Fig. 2

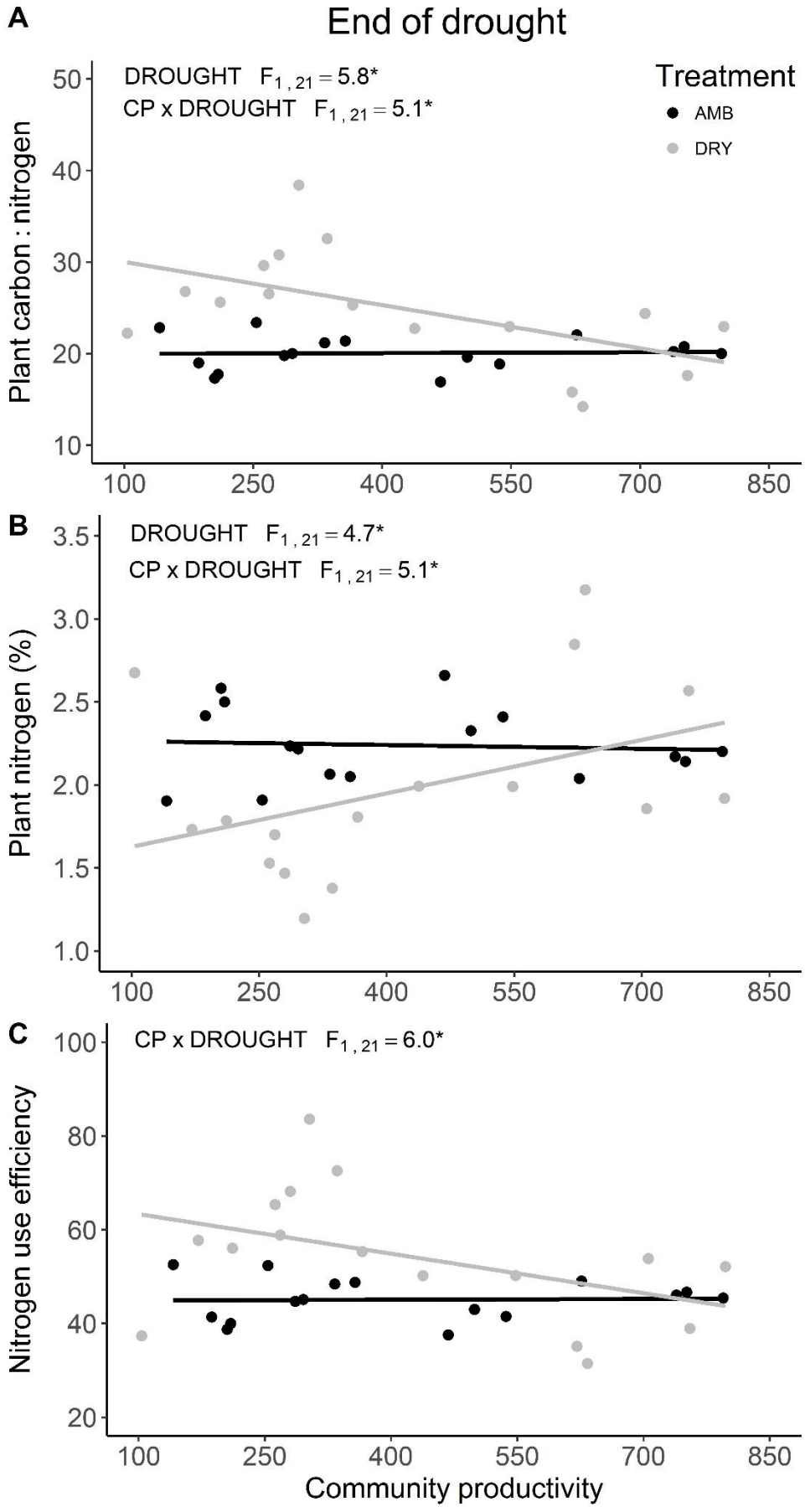

Fig. 3

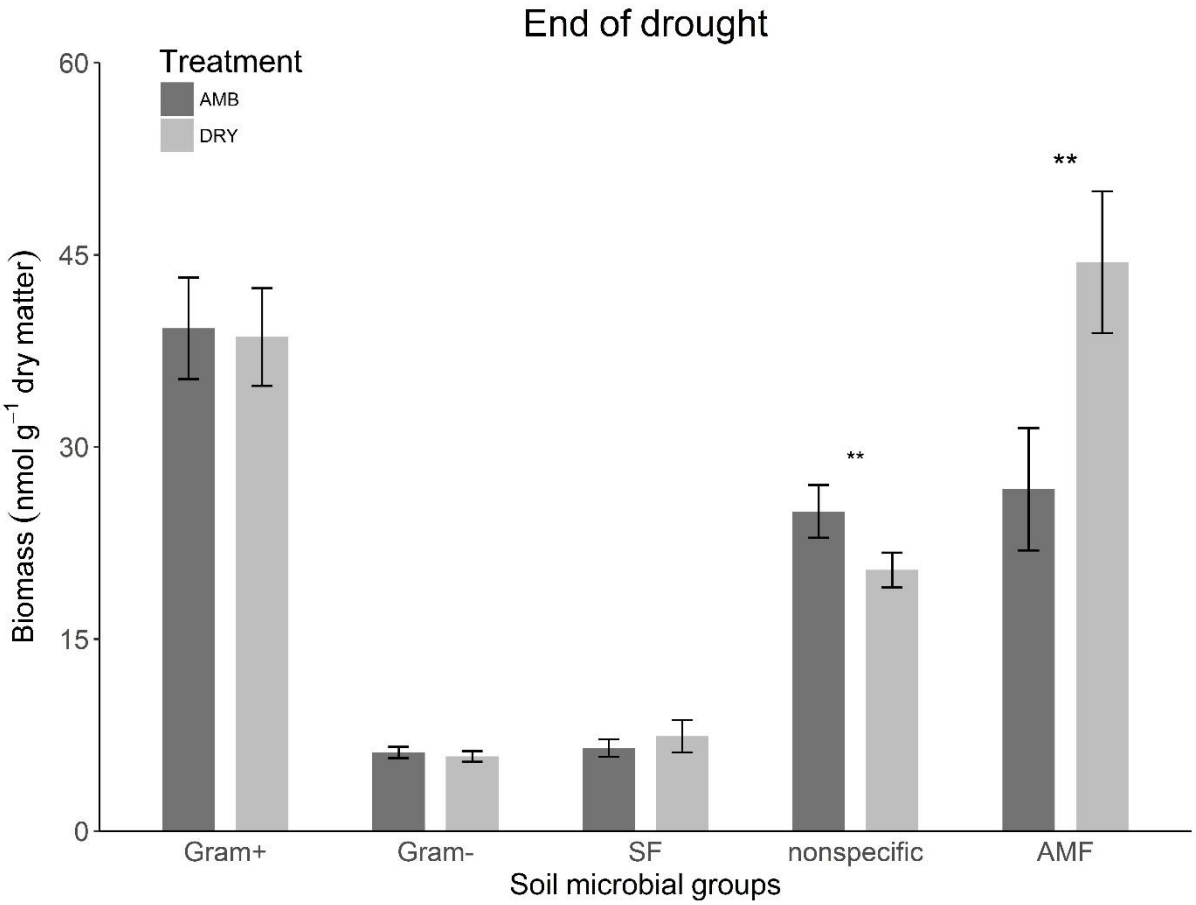

Fig. 4

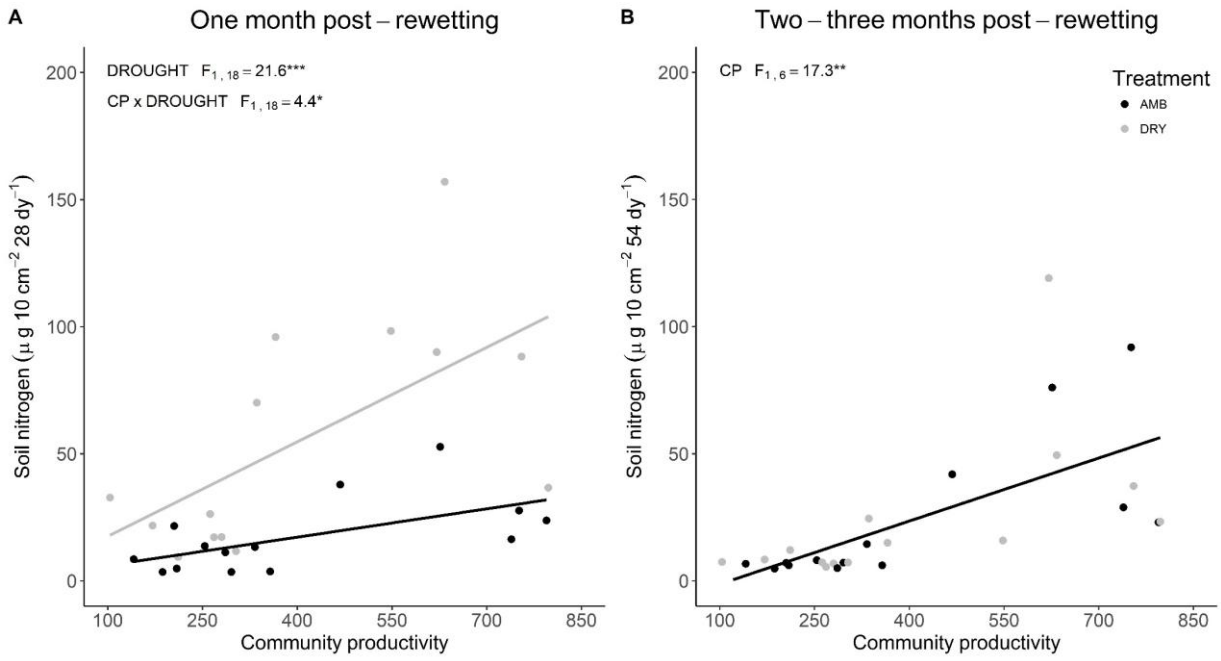

Fig. 5

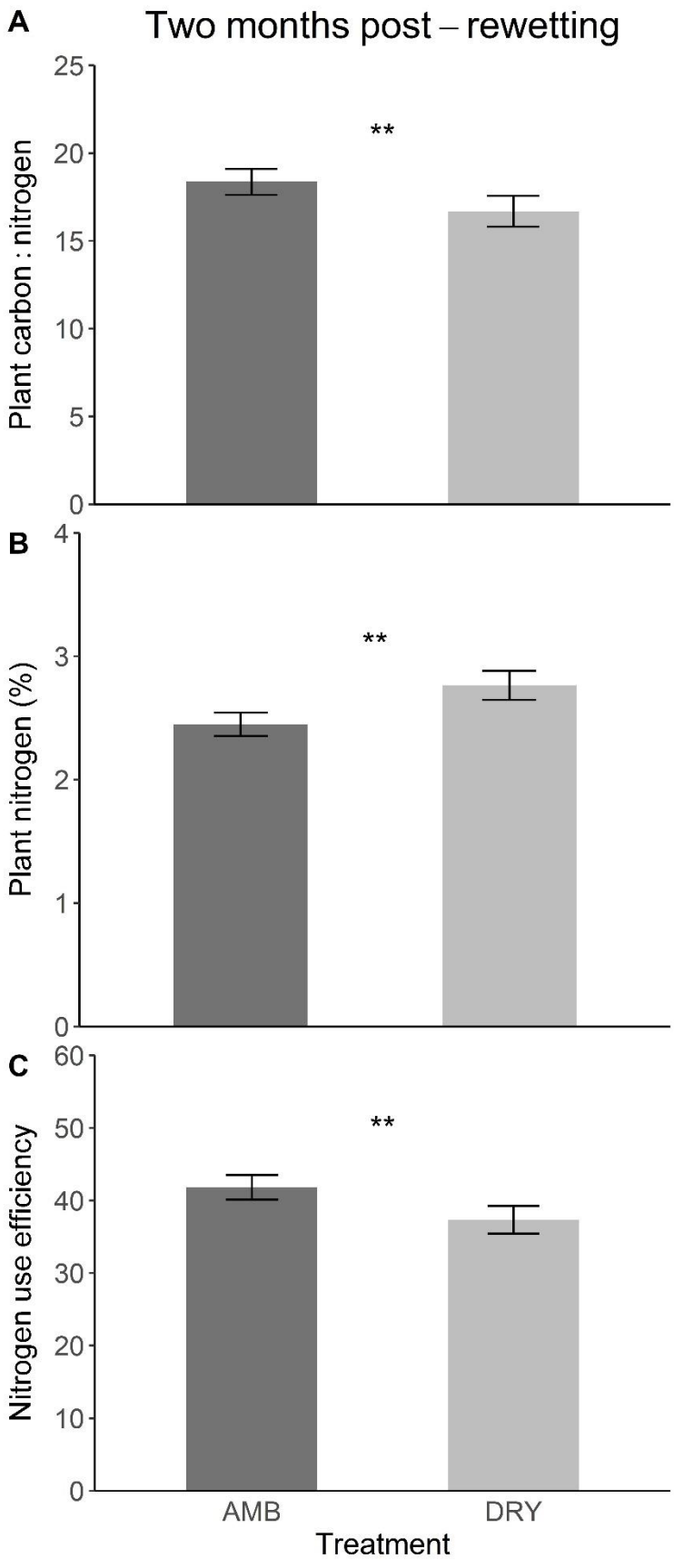

Fig. 6