**Soil microbial community resistance to drought and links to C stabilization in an
Australian grassland**

Alberto Canarini^{1*}, Yolima Carrillo², Pierre Mariotte^{1,3}, Lachlan Ingram¹, Feike A. Dijkstra^{1*}

¹ Centre for Carbon, Water and Food, Faculty of Agriculture and Environment, School of Life
and Environmental Sciences, The University of Sydney 380 Werombi Road, Camden NSW 2570
Australia

² Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797
Penrith NSW 2751 Australia

³ Ecole Polytechnique Fédérale de Lausanne (EPFL), School of Architecture, Civil and
Environmental Engineering (ENAC), Laboratory of Ecological Systems (ECOS), Station 2, 1015
Lausanne, Switzerland.

* Corresponding authors:

Alberto Canarini, School of Life and Environmental Sciences, The University of Sydney, Centre
for Carbon, Water and Food, 380 Werombi Road, Camden NSW 2570, Australia.

Tel.: +61 2 9351 1892

Email: alberto.canarini@hotmail.it

Abstract

Drought is predicted to increase in many areas of the world, which can greatly influence soil microbial community structure and C stabilization. Increasing soil carbon (C) stabilization is an important strategy to mitigate climate change effects, but the underlying processes promoting C stabilization are still unclear. Microbes are an important contributor of C stabilization through the adsorption of microbial-derived compounds on organo-mineral complexes. Management practices, such as addition of organic amendments might increase soil C stock and mitigate drought impacts, especially in agro-ecosystems where large losses of C have been reported.

Here, we conducted a drought experiment where we tested whether the addition of organic amendments mitigates drought effects on soil C stabilization and its links to microbial community changes. In a semi-natural grassland system of eastern Australia, we combined a management treatment (compost vs. inorganic fertilizer addition) and a drought treatment using rainout shelters (half vs. ambient precipitation). We measured soil moisture, soil nitrogen and phosphorus, particulate organic C (Pom-C) and organo-mineral C (Min-C). Microbial community composition and biomass were assessed with PLFA analyses. A structural equation modeling (SEM) approach was used to examine the controls of soil moisture, Pom-C and nutrients on soil microbial biomass and community structure and changes in Min-C.

Overall, the drought treatment did not affect microbial community structure and Min-C, while fertilizer only marginally increased Min-C, highlighting the resistance to these treatments in this grassland soil. In the surface soil (0-5 cm) Min-C was strongly associated with fungi that may have been stimulated by root exudates, and by gram-negative bacteria in the deep soil (5-15 cm) that were more affected by Pom-C and soil moisture. .

We conclude that the grassland microbial community and its effect on Min-C at our field-site were non-responsive to our drought treatment, but sensitive to variability in soil moisture and microbial community structure. Our findings also show that surface compost application can moderately increase soil C stabilization under drought, representing a useful tool for improving soil C stability.

Key words: reduced precipitation; organo-mineral carbon; compost; rain out shelter; path-analysis; grassland.

1. Introduction

Climate change can have severe consequences for natural and agro-ecosystems and there is particular concern of how climate change will affect soil organic carbon (SOC) pools. Soil contains the largest pool of C in most terrestrial ecosystems (Scharlemann et al., 2014), and small changes in SOC can cause large changes in the concentration of atmospheric CO₂, the most abundant greenhouse gas. Global warming is affecting precipitation and the hydrological cycle (Dai, 2011; Min et al., 2011; Trenberth et al., 2014), possibly causing more intense drought periods in large parts of the world (Handmer et al., 2012). Changes in amount, intensity and frequency of precipitation greatly affect processes that govern SOC pools and dynamics (Canarini and Dijkstra, 2015; Cleveland et al., 2010; Fuchslueger et al., 2014; Zhang et al., 2013; Zhang et al., 2015). For example, drought has shown the ability to turn grassland ecosystems from C sinks into C sources (Hoover and Rogers, 2016; Zhang et al., 2010; Zhang et al., 2011). Of particular relevance is C stabilization, where different SOC pools possess a wide range of

degrees in stability and consequently turnover times (Schmidt et al., 2011), ultimately regulating C emissions. However, information about drought effects on soil C stabilization is still lacking.

Microbial derived products and organo-mineral interactions play a central role in soil C stabilization (Ahrens et al., 2015; Cotrufo et al., 2013a; Feng et al., 2014; Six et al., 2006). Organo-mineral interactions, by protecting organic matter from different mineralizing agents, can increase the mean residence time of C up to millennia (Jones and Singh, 2014). Sorption of dissolved organic carbon (DOC) on organo-mineral complexes is not saturated in most soils (Feng et al., 2014), leaving potential for the mineral pool to act as a C sink. It has been suggested that a major component of organo-mineral protected C originates from microbial biomass (Solomon et al., 2012), which suggests a close link between microbial residues and C stabilization in soil (Liang et al., 2011; Schurig et al., 2013). In general, the prevailing view is that fungal-dominated communities can sequester more C, by a higher growth efficiency, and produce more recalcitrant cell walls compared to bacteria (Bailey et al., 2002; Six et al., 2006). However, little information is available on how the individual microbial groups are correlated with the organo-mineral fraction (Gude et al., 2012).

Variation in soil moisture could modulate the transfer of microbial necromass C to organo-mineral complexes by changing biomass composition and community structure of important microbial groups involved in C sequestration. For instance, drought can reduce stabilization of fungal dead mass through changes in fungal metabolites composition (Crowther et al., 2015). Recently, we showed a positive relationship between the amount of plant-derived C in microbial biomass and plant-derived C associated with organo-mineral complexes, where drier soil conditions reduced the transfer of C from wheat plants to microbial biomass primarily due to a reduction in plant biomass (Canarini and Dijkstra, 2015). Therefore reduced soil water

availability could reduce the formation of organo-mineral complexes through direct (reduced microbial activity) and indirect effects (reduced C transfer from plants) on microbes. However, effects on microbial communities are inconsistent. A few studies showed a relative increase in fungi and gram-positive bacteria under drought (Barnard et al., 2013; Fuchslueger et al., 2014; He et al., 2012; Maestre et al., 2015; Vries et al., 2012) confirming findings from laboratory incubations (Manzoni et al., 2012; Schimel et al., 2007), while other field studies observed limited or no effects on microbial community composition (Landesman and Dighton, 2010; McHugh and Schwartz, 2014; Rousk et al., 2013). This suggests that drivers other than a simple reduction in soil moisture may be required to explain the effect of drought on microbial communities.

As the effect of drought on microbes may be dependent on nutrient and C availability, fertilization practices may have strong impacts on this process, especially considering the wide spread use of fertilizers in agricultural systems. The application of fertilizers, either in mineral form or as organic amendments, can have important effects on microbial community composition and soil processes. While fertilizer application commonly increases plant productivity, the effect of nutrient supply on plant yield can be strongly reduced under water limitation (Graciano et al., 2005). In addition, mineral fertilizers, especially in the form of nitrogen (N), can reduce microbial biomass and activity (Tian et al., 2014; Treseder, 2008; Wallenstein et al., 2006; Yang and Zhu, 2015). On the other hand, organic amendments, such as compost, can increase microbial biomass and activity compared to mineral fertilizers during water limited periods (Sahs and Lesoing, 1985). Indeed, compost can increase soil organic matter content, which improves water retention in the soil (Diacono and Montemurro, 2010; Hueso et al., 2012). Hueso et al. (2012) found that the addition of compost increased the resistance

(insensitivity to disturbance) of the microbial community to a 6-months drought period compared to unamended soils. Organic amendments may therefore represent a way to alleviate adverse effects of drought on yield and microbial communities, and at the same time increase C storage in soil.

Fertilizers (either inorganic or organic) in pasture systems are generally applied to the surface, likely limiting their effects to the topsoil. There are relevant differences in resources availability (*i.e.*, available C substrates) and microbial communities between different soil depths (Fierer et al., 2003). Therefore, it is important to understand whether surface application of organic amendments can increase resources also in deeper soil layers, and mitigate drought effects where substrates availability is more limited. Moreover, since microbes can change their C use efficiency with depth (Spohn et al., 2016), C stabilization mechanisms could be led by different drivers, resulting in different interactive effects of drought and fertilizer application with depth.

The aim of this study was to evaluate the effects of drought on microbial community composition and soil C stabilization and to further investigate the relationship between microbial groups and soil C stabilization in a semi-natural grassland. For this, we examined the interactive effects of two management practices involving addition or no addition of C (compost *vs.* mineral fertilizer addition), and drought (ambient *vs.* half of the precipitation) using rainout shelters, in a full factorial design. Fertilizer management and drought effects on soil C (particulate organic and organo-mineral C) and microbial communities were examined for two different depths (0-5 and 5-15 cm). We hypothesized that compost will increase organo-mineral C and the biomass of the total microbial community, compared to mineral fertilizer. We predicted that this difference would be stronger under drought conditions, because compost can increase water retention and

energy supply from organic products. To examine drought and fertilizer management effects on the microbial community structure and C stabilization, we used a structural equation modeling (SEM) approach. Soil moisture, nitrate (NO_3^-) and particulate organic C were used as continuous moderators to find relationships with the abundance of specific microbial groups and the pool of organo-mineral C. We expected that variation in the biomass of the microbial community caused by variation in soil moisture and particulate organic C would be positively related to the organo-mineral C pool. Since fungi are generally more resistant to drought conditions and more efficient at sequestering C, we hypothesized that fungi would correlate more strongly to the organo-mineral C pool.

2. Materials and methods

2.1 Site description and experimental design

This study was conducted in a semi-natural grassland at John Bruce Pye Farm on the campus of The University of Sydney, NSW, Australia (33°55'51"S, 150°39'38"E). The site is at 81 m above sea level and has a maximum temperature of 29.8°C in January (Summer) and 4.1 °C as minimum temperature in July (Winter). The mean annual precipitation recorded over the last 20 years is 692 mm, ranging between 414 and 1041 mm (Bureau of Meteorology, 2016). Vegetation is dominated by C4 grasses (*Paspalum dilatatum*, *Paspalum distichum*, *Cyperus brevifolius*, *Setaria incrassata*, *Sporobolus africanus*) and a C3 grass (*Microlaena stipoides*). In the 0–15 cm depth, approximately 85% ($\pm 7\%$) of the root biomass was in the first 0-5 cm and 15% ($\pm 7\%$) in the 5-15 cm increment. The soil is a clay loam and classified according to the Australian Soil Classification (Isbell, 2002) as a red-brown Chromosol. Average values for soil characteristics in the top 15 cm are: clay 35%, sand 34%, silt 31%, organic C% 3.2, N% 0.23, P% 0.015, pH 5.7

(H₂O). Cattle previously grazed the area, but were excluded during the experiment. However, grazing was simulated by clipping plant biomass in plots and mowing the area around plots twice a year (May and January).

In January 2014, we selected an area (23 x 17 m) of uniform grass density and established 16 plots (2 × 2 m) in a block design containing 4 blocks of 4 plots. We explored the influence of drought and fertilization management on soil microbial communities and organo-mineral C in a full factorial experiment including 4 treatments randomly assigned in plots within each block: reduced precipitation and compost addition (Red-CP), ambient precipitation and compost addition (Amb-CP), reduced precipitation and mineral fertilizer addition (Red-MN), or ambient precipitation and mineral fertilizer (Amb-MN). The area is on a 10% slope with a northerly aspect and we accounted for the slope by placing blocks, parallel to the slope. In order to simulate drought, rainout shelters (2 m width × 2 m length × 1m height) were constructed following the design of Yahdjian and Sala (2002). Clear acrylic gutters (transmitting almost all of the visible light) were used to intercept half of the ambient precipitation. Intercepted precipitation was diverted away from the plots with separate gutters. A plastic barrier was buried 40 cm into the ground at the side of each plot facing the slope to prevent runoff and lateral flow going into the plot. To control for shelter effects on UV light interception, shading and temperature, the same shelters were installed in control plots, but mounted upside down in order to let all precipitation through. Compost and mineral fertilizers were uniformly applied to the surface in May 2014 after the aboveground plant biomass clipping. The compost, derived from domestic municipal waste, was applied at a rate of 6 t ha⁻¹ and contained 28% C, 1.9% N, 0.46% P, 0.9% K, 3.4% Ca and 0.3% S (or a total of 114 kg ha⁻¹ N, 28 kg ha⁻¹ P, 54 kg ha⁻¹ K, 204 kg ha⁻¹ Ca, and 18 kg ha⁻¹ S). The mineral fertilizer was applied at a rate of 40, 10, 16, 60, 10 kg ha⁻¹

respectively for N, P, K, Ca and S (from urea, potash and superphosphate). The nutrients applied with compost were higher than the mineral fertilizer applied, because we expected not all nutrients to be mineralized in the compost. Also, both fertilizers (mineral and compost) rates were based on standard practices for these grasslands.

Sampling only occurred in the central one meter square subplot, while the surrounding area (50 cm wide on all four sides) was a buffer zone. Temperature (S-TMB-M0XX) and moisture probes (S-SMx-M005) were installed at 5 cm soil depth in each subplot of the first and last block on the 15th of April 2014, and temperature and moisture were recorded every 15 minutes on a data logger (HOBO U30 station, Onset Computer Corporation, Bourne, MA, USA). Other meteorological data were obtained from the Badgerys Creek meteorological station (Bureau of Meteorology, 2016) located about 7 km from the experimental site.

2.2 Soil collection, soil moisture and available C, N and P

Soil samples were collected in November 2014, December 2014 and January 2015 (one year after manipulations were initiated) in each subplot and at two depths: 0-5 cm (surface), and 5-15 cm (deep). Three soil cores (2.5 cm in diameter) were collected and pooled together by depth before analysis, for a total of 96 samples. Soil samples were sieved (2 mm) and homogenized on site, and subsampled for phospholipid fatty acids (PLFA) analysis, nutrients and gravimetric water content measurements. Subsamples for PLFA analysis were immediately frozen with a portable freezer, freeze-dried within 2 weeks from harvest and then kept at -20°C until analysis. The rest of the soil was brought to the laboratory, kept at 4°C and analyzed the day after harvesting.

Soil samples were analyzed for water gravimetric content by drying the soil at 65°C for 72 hours. Dissolved organic carbon (DOC) and dissolved nitrogen (DN) were extracted by shaking samples with 0.05 M K₂SO₄ for an hour at 4:1 solution to soil ratio, and then filtered through Whatman #42 filter paper. Extracted solutions were measured for C and N content on a TOC-N analyzer (Shimadzu, Kyoto, Japan), and for NO₃⁻ and NH₄⁺ on a Flow Injection Analyser (FIA automated ion analyzer, Lachat Instruments, Loveland, CO, USA). Available phosphorus (P) was extracted with 0.03 M NH₄F – 0.025 N HCl. The P concentration was measured colorimetrically using the ammonium molybdate–stannous chloride reagent (Olsen and Sommers, 1982).

2.3 Soil C fractionation

Dry soil samples were analyzed for total C and N using a CHN analyzer (LECO TruSpec CHN, USA). Soil samples were separated into a soil particulate organic matter fraction (Pom-C) and a mineral fraction (Min-C) by dispersion in sodium hexametaphosphate and following sieving using a 53 µm sieve (Canarini and Dijkstra, 2015). After fractionation, soil samples were dried, ground and analyzed for total C and N using a CHN analyzer (LECO TruSpec CHN, USA).

2.4 Soil PLFA

Soil PLFAs were extracted and analyzed using the high throughput method developed by Buyer and Sasser (2012). Briefly, around 2 grams of freeze-dried soil were used for Bligh–Dyer lipid extraction (Bligh and Dyer, 1959). A stream of N₂ was used for drying the different phases. All samples were dried and analyzed by GC following trans-esterification for quantitative analysis relative to an internal standard. Gas chromatography was performed on an Agilent 7890A GC

(Agilent Technologies, Wilmington, DE, USA) and FAME profiles were identified using the MIDI PLFAD1 calibration mix and the software SHERLOCK version 6.2 (MIDI, Inc., DE, USA).

For each sample, the abundance of individual fatty acid methyl esters was expressed as $\mu\text{mol PLFA g}^{-1}$ dry soil. Gram-positive bacteria were represented by PLFAs i15:0, a15:0, i16:0, a17:0, and i17:0 (Nie et al., 2013; Zelles, 1997), gram-negative bacteria by 14:0 2OH, 16:0 2OH, 18:1 ω 5c, 18:1 ω 7c, 16:1 ω 7c, i17:1 ω 9c, 17:1 ω 8c and cy19:0 (Brockett et al., 2012; Liu et al., 2012; Zelles, 1999), unclassified bacteria by 14:0, 15:0, 17:0 (Frostegård and Bååth, 1996; Lindahl et al., 1997), actinomycetes by 10Me16:0, 10Me17:0, 10Me17:1 ω 7c, 10Me 18:0 (Bell et al., 2009; Frostegård and Bååth, 1996); (Brockett et al., 2012; Kujur M. and K., 2014), saprotrophic fungi by 18:1 ω 9c, 18:2 ω 6c, 20:1 ω 9c (Kao-Kniffin and Zhu, 2013; Li et al., 2015; Nelson et al., 2011; Nie et al., 2013), arbuscular mycorrhizal fungi (AMF) by 16:1 ω 5c (Brockett et al., 2012; Nie et al., 2013; Olsson, 1999) and protozoa by 20:4 ω 6c, 20:5 ω 3c, 20:3 ω 6c. Other PLFAs such as 14:0, 16:0 and 18:0 were considered as unclassified PLFAs (Brockett et al., 2012; Ruess and Chamberlain, 2010) and also used for analysis of the microbial community. Fungal to bacterial ratio (F/B) was calculated by dividing fungal PLFAs (not including AMF) by the sum of all bacterial PLFAs.

2.5 Data analysis

A four-way repeated measures analysis of variance (ANOVA) was used to test for main and interactive effects of fertilizer management (FM), drought (DR), time and depth on soil moisture, NH_4^+ , NO_3^- , available P, DOC, DON, Pom-C and Min-C fractions, and individual microbial groups with block as error term. We did not include unclassified PLFAs as they are not

indicative of a specific microbial group, or the PLFA 16:1 ω 5c that has been associated with AMF, because it has also been found in other microbial groups (Frostegård et al., 2011). ANOVAs were performed in JMP v. 8.0.1 (SAS Institute, Cary, NC, USA). We compared Euclidean distances of microbial communities using permutational multivariate analysis of variance (PerMANOVA) to determine effects of experimental treatments, time and soil depth on the structure of microbial communities. Block effect was included as random term. Data were further analyzed by redundancy analysis (RDA, vegan-R package; Oksanen et al., 2011). This constrained ordination method provides a multivariate regression model for the relationship between microbial community and soil characteristics, where constraints include continuous variables. We tested all soil parameters, allowing for the inclusion of soil moisture as a proxy for drought and Pom-C and nutrients as proxies for fertilization management. The significance of each explanatory variable on microbial PLFA was tested using 999 permutations. Block effect reflecting the slope was also included in the analysis. The functions ‘decostand’, ‘rda’ and ‘RsquareAdj’ in the package ‘vegan’ were used to test for multivariate homogeneity, RDA and R^2 value corrections, respectively (Oksanen et al., 2015). The PerMANOVA and RDA statistical analyses were performed in the R language environment (R Core Team, 2013).

Significant results from ANOVA were linked using *a priori* knowledge in a path analysis model in order to determine the drivers of microbial community structure and linkages with organo-mineral C. Path analysis represents a special class of structural equation modeling (SEM) containing only observed variables, where SEMs are probabilistic models that can include multiple predictors and response variables in a single causal network. The path analysis was performed using the software MPlus version 6.1 (Muthén and Muthén, 1998-2010). Based on an *a priori* knowledge a full model was built including all hypotheses (for full list of *a priori*

hypothetical pathways see Supplementary information, Fig. S1). As depth resulted in significant differences for soil variables and microbial community, we chose to build two separate models, one for the surface soil and the other for the deeper soil layer. Significant full models were simplified by step-wise exclusion of non-significant variables (either by weights or covariance) as estimated by AIC (Akaike information criterion), until a minimal adequate model was reached (Milcu et al., 2013). The relationship between microbial community composition with Min-C was also investigated, as previous literature suggested links between these two variables (Baldock and Skjemstad, 2000; Cotrufo et al., 2013a; Liang et al., 2011; Solomon et al., 2012). Because biodiversity or evenness indices are not suitable with PLFAs (Frostegård et al., 2011), we chose the F/B ratio as a common index for soil C processes (de Vries et al., 2006). The sum of total PLFA groups was chosen to represent an index of the microbial community biomass. The adequacy of the models was determined by non-significant chi-square tests (χ^2 , $P > 0.05$), low root mean square error of approximation index (RMSEA < 0.1), Tucker-Lewis Index (TLI ≥ 0.90) and high comparative fit index (CFI ≥ 0.90) (Grace et al., 2010). Soil moisture, Pom-C and nutrient concentrations were considered as proxies for drought and fertilizer management treatments. In order to correct for time dependency, time was nested within plots through the option CLUSTER.

3. Results

3.1 Drought and management effects on soil nutrient and carbon fractions

During the experimental period (Jan 2014 – Jan 2015), the area received 824 mm of rain, with much of the rainfall occurring during August-September 2014 and December 2014 (Fig. 1). Over the experimental period we observed a faster decrease in soil moisture (volumetric soil

moisture measured with soil moisture probes) after rain events in reduced precipitation compared to ambient precipitation plots, while soil moisture in plots with compost addition tended to be higher during dry periods. The gravimetric soil moisture measured in soil samples collected in November 2014, December 2014, and January 2015 was on average significantly lower in reduced precipitation plots at both depths ($P < 0.001$, Table 1, Fig. 2). Gravimetric soil moisture was not significantly different between compost and mineral fertilizer amended plots.

Inorganic N ($\text{NO}_3^- + \text{NH}_4^+$) concentrations ranged from 2 to 27 mg kg^{-1} of dry soil and significantly increased with drought in both soil depths, especially for NO_3^- (Table 1), but no differences were found between compost and mineral fertilizer addition. Available phosphorus (P) was not affected by the drought or by the fertilization management. Overall neither Pom-C and Min-C were affected by drought or fertilizer treatment (Table 1), however there were some significant interaction terms. Specifically, Pom-C increased in the surface soil with compost addition, but only under drought causing a significant drought $\times$ fertilization management $\times$ depth interaction ($P = 0.02$). Compost addition also increased Min-C in the surface soil (fertilizer management $\times$ depth interaction, $P = 0.04$). There were no drought or fertilization management effects on Pom-C and Min-C in the deep soil. In most cases, significant time effects were observed for all soil parameters, indicating seasonal variations (Table 1), but interactions between time and drought or fertilizer management treatments were rare.

3.2 Microbial community composition and structure

The drought and the fertilization management treatments did not significantly impact microbial biomass (total or divided by group) and the fungal to bacterial ratio (Table 2). Depth significantly decreased the abundance of all microbial groups (Fig. 3, Table 2), but did not

impact the F/B ratio. Time effects were rare, and interactive effects of time and treatments were mostly not significant.

Microbial community as a whole was initially analyzed with PerMANOVA, both on individual PLFAs and microbial groups, but we did not find any significant treatment effects (Table S1 and S2). Soil depth and time effects were always significant, but treatment interactions were almost never significant. However, the experimental set up and sampling created gradients in soil parameters (including moisture, Pom-C and NO_3^-), possibly masking the treatment effects, even when correcting for the block effect (*e.g.*, error term).

We then performed a redundancy analysis to explore the effects of continuous variables on individual PLFAs (Fig. S3). Results from RDA were similar to PerMANOVA. Specifically, Axis 1 explained most of the variability (20.95%) while Axis 2 explained 6.46%. Soil depth was strongly associated with Axis 1 and negatively correlated with Pom-C and available P, while time and soil moisture were associated with Axis 2.

3.3 Linkages between microbes and soil Min-C

We investigated interactions between soil parameters and soil microbial biomass and decomposer groups and their impacts on Min-C, which is considered as one of the most stable pools of carbon in soil. Based on *a priori* knowledge (Fig. S1), we identified soil moisture, Pom-C and nutrient concentrations as important drivers affecting microbial community composition and biomass, and microbial community as an important vector of the increase in organo-mineral C (Min-C). Soil moisture, Pom-C and nutrients are parameters that were manipulated in our experimental set up (precipitation reduction, compost and mineral fertilizer addition). After step-

wise exclusion of non-significant variables, out of all nutrients, only NO_3^- remained as a significant explanatory variable.

The fitting parameters of all minimal adequate path analysis models (SEM) were excellent (Fig. 4 and 5), and models explained 22% (surface soil) and 51% (deep soil) of the variance in Min-C. In the surface soil (Fig. 4a), Min-C increased with increasing microbial biomass and Pom-C, and with greater abundance of fungi relative to bacteria (F/B ratio), but was not affected by soil moisture. We used covariance (*i.e.*, no implied causality) to test the relationship between NO_3^- and microbial biomass, as soil N can affect microbial biomass but microbes can also affect soil N through mineralization. NO_3^- was negatively correlated with total microbial biomass and fungal to bacterial ratio. In deep soil (Fig. 4b), an increase in total microbial biomass was associated with an increase in Min-C, which was likely indirectly driven by an increase in soil moisture and Pom-C. Pom-C also had highly significant direct correlation to Min-C in the deep soil. Nitrate was negatively affected by soil moisture but this yielded limited effect on the microbial biomass.

We further used SEM to specifically identify effects of the different microbial groups and interactions between groups. Relationships between microbial groups were tested with covariance, as all groups can affect one another through competition for resources. For both the surface and the deep soil, the models highlight strong positive correlations between all microbial groups, indicating limited competition within groups. In the surface soil (Fig. 5a), we identified fungi and Pom-C as direct drivers of Min-C increase (path = 0.22 and 0.41, respectively), while soil moisture had no effects. Pom-C also had a significant positive relationship with gram-negative bacteria while fungi and NO_3^- were negatively correlated. In the deep soil (Fig. 5b), soil moisture became more important and benefited gram-positive and -negative bacterial by

increasing their biomass. Pom-C also positively affected gram-positive and -negative bacteria. These effects on gram-negative bacteria indirectly increased Min-C (standardized direct path coefficient: 0.36, $P < 0.001$). Increasing Pom-C also had direct positive effects on Min-C and fungi by increasing their abundance. Specifically, the standardized direct path coefficient between Pom-C and Min-C doubled compared to surface soil (Fig. 5), although fungi did not significantly correlate with Min-C in the deep soil.

4. Discussion

In our study, we tested the effects of drought, simulated as a constant reduction in precipitation, on microbial community composition and structure and on the Min-C pool. We also tested whether compost application could increase soil mineral C compared to the standard mineral fertilizer application used in Australian agro-ecosystems, especially in drought plots.

4.1 Treatment effects

In our grassland site, drought did not have a strong effect on the microbial community composition nor on the main microbial decomposer groups. While the experiment successfully reduced soil moisture content, periods of continuous large precipitation events (*e.g.*, August-September or late December 2014) caused soil moisture saturation in both the reduced and ambient precipitation treatments (Fig. 1), and thus dry-rewetting cycles associated with our drought manipulation might have buffered the effects on microbial communities, as previously shown (Hamer et al., 2007; Kaisermann et al., 2015). Nevertheless, our findings show that the main microbial groups were relatively stable to short time changes in soil moisture and these are in accordance with previous experiments showing strong resistance of grassland soil food webs

to drought manipulations (de Vries et al., 2012; McHugh and Schwartz, 2014). We also showed that drought effects on Min-C (the stable pool of C) were not significant, further support that grassland soil processes tend to be resistant to climate change (Hoover and Rogers, 2016).

Fertilization management also had no effect on the main microbial groups or on the overall microbial community biomass. However, both fertilizer types were applied 6 months before the first soil sampling, possibly indicating that measurable effects may be short-lived. Pom-C increased significantly with compost addition in drought plots but not in ambient precipitation plots (Table 1), suggesting that a substantial amount of compost may have decomposed in plots with higher soil moisture since the application date. Compost addition also significantly increased Min-C in the surface soil, suggesting that part of the added compost was converted into Min-C.

4.2 Differences in depth

Despite non-significant treatments effects on microbial community, soil depth significantly affected the community composition. Similarly to our study, it was shown that, in deeper soil, the relative abundance of gram positive increased while fungi and gram-negative bacteria decreased (Fierer et al., 2003; Huang et al., 2011). Fierer et al. (2003) explained these differences mainly through changes in resources availability (C). The same pattern was observed in our experiment, where microbial community separation by depth was explained by a negative correlation with Pom-C and available P (Fig. S3). Variation in microbial community with soil depth may also have been affected by a greater supply of energy through root exudation in the surface soil (Lamb et al., 2010; Mariotte et al., 2015).

4.3 Linkages between microbial community and Min-C by depth

Our data also showed large variation between the plots, indicating that a high degree of spatial variability in the investigated soil variables was evident, which could have masked potential effects of drought and fertilizer management. In order to account for this variation and to show linkages between microbial community and Min-C, we used a path analysis (SEM) where investigated soil biotic and abiotic measures were linked together as continuous variables. Both depths revealed a positive correlation between Min-C and microbial community biomass, confirming previous studies showing the importance of microbes for the formation of organo-mineral complexes (Cotrufo et al., 2013b; Liang et al., 2011; Solomon et al., 2012), although relationships between microbes and other soil variables differed between soil depths.

In the surface soil, we did not find relationships between Pom-C or soil moisture and total microbial community biomass or composition. Soil organic matter, and in particular Pom-C, is considered one of the main forms of energy that microbes utilize (Schimel and Schaeffer, 2012), and was correlated with microbial biomass in different arable soils (Anderson and Domsch, 1989). However, in the surface soil of our grassland it may not represent the main energy source, while plants, through root exudates (or rhizodeposition), could provide the main supply of C to microbes (Merino et al., 2015; Schmidt et al., 2011). In our experiment root biomass was mostly observed in the surface soil (more than 80%), similarly to other grassland experiments (Carrillo et al., 2014; Liu et al., 2016). In a previous study, we also found a linear relationship between the amount of plant-derived C in microbes and in organo-mineral complexes, indicating the importance of rhizodeposition for the formation of organo-mineral complexes mediated by microbes (Canarini and Dijkstra, 2015). Therefore, we speculate here that the variation in total microbial community biomass unexplained by our model was caused by rhizodeposition.

However other factors shaping the microbial community that we did not consider may also have played a role (*e.g.* plant community, macro-fauna). The main group correlating to Min-C was fungi, which explains the positive correlation between the F/B ratio and Min-C. Rhizodeposits may have been an important C source of fungi (Pausch et al., 2016; Scheunemann, 2015), especially during drought (Fuchslueger et al., 2014), which could explain the lack of a correlation between Pom-C and fungi. We further found a negative correlation between NO_3^- and fungi. Mineral fertilizers quickly release nutrients in the soil, while compost has a slower rate of nutrient release through decomposition. Possibly, a negative effect of mineral fertilizer on fungi, at the time of fertilizer application, may have contributed to the significant effect of compost on Min-C. However, a causal relationship between NO_3^- and fungi cannot be established as fungi could have been reduced by N or *vice versa*.

In the deeper soil layer, we found clear effects of soil moisture and Pom-C on microbial community. Deeper soil microbes largely depended on Pom-C, because alternative C sources were most likely limited (*i.e.*, rhizodeposition). With reduced rhizodeposition, soil moisture can play a more important role for microorganisms by controlling mobility of substrates (Schimel et al., 2007). It was shown that microbial sensitivity to drought increased with reduced C inputs from plants (de Vries and Shade, 2013). Our results show that in the deep soil, where C resources are relatively scarce and plant inputs limited, Pom-C and soil moisture have strong controls over microbial biomass. Min-C increased with more Pom-C, directly and indirectly, through gram-negative bacteria. Gram-negative bacteria were also the major contributors of organo-mineral complexes in two other agricultural soils (Gude et al., 2012). While this group of bacteria is generally considered as fast growing, competing for simple organic compounds, it is equally able to utilize complex organic compounds (You et al., 2014). Thus gram-negative bacteria can be

considered as more generalist organisms adaptable to varying availability in resources. Therefore, the potential for high turnover rates coupled with a wide plasticity in substrate utilization, could potentially result in large amounts of microbial necromass that may play an important role in the formation of Min-C (Kindler et al., 2006). In a recent study, gram-negative bacteria (*Escherichia coli*) labeled with ^{13}C were introduced in soil and 33% of the biomass remained in the non-living SOM pool after 224 days (Kindler et al., 2009). Because microorganisms differ in their cell structures and physiological functioning, the presence of specific microbial groups could be important for the formation of Min-C. In our study, fungi were found to be important for Min-C in the surface soil, while gram-negative bacteria in the deeper soil. Thus, any treatment causing changes in microbial community composition could potentially change the formation of stable C pools, although further studies are needed to confirm this speculation.

While our results indicate a strong link with microbial community and Min-C, results have to be interpreted cautiously. Indeed path analysis is very useful in showing linkages and indicating mechanisms behind the investigated processes, but linear relationships are not a proof of how microbial biomass contributed to organo-mineral C, while other types of experiments (*e.g.* use of isotope tracers or necromass biomarkers) should address this specific quantification.

5. Conclusion

We showed that soil microbial community was highly resistant to drought in our grassland experiment. The drought treatment did not affect mineral-organic C either, suggesting that grassland soil C stabilization may not be affected by drought, at least not within a relative short time, as in our experiment (one year). Compost addition had limited positive effects on

Min-C (as compared to mineral fertilizer), and the increase in Min-C was restricted to the surface soil.

We showed that the microbial community biomass was strongly positively correlated to Min-C, although abiotic controls over microbial community biomass and structure, and Min-C differed for the two soil depths. As a result, fungi in surface soil and gram-negative in deeper soil showed the strongest relationships with Min-C. Future experiments should quantify the precise transfer of biomass from microbes to Min-C, to confirm our results.

In conclusion, organic amendments such as compost can moderately enhance the amount of stable C through microbial processing, and microbial biomass is strictly correlated to Min-C. Grassland soil microbial community is resistant to drought and thus Min-C, which is linked to microbial community, was not affected. However, the path analysis showed that water and particulate organic C significantly modulate Min-C increase in deeper soil layers that are more C limited, indicating that drought treatment effects may have been masked by the intense variability in soil moisture at our field experiment.

Acknowledgements

We thank Paul Lipscombe, Thalles Reis, Michael Willian, Luca Evangelisti, Gabriele D'Alonzo and Maggie Norton for field assistance, Jamie Maclean and Hero Tahaei for laboratory assistance, and Duncan Le Good of Suez Recycling and Recovery for Mixed Source Compost supply. This research was supported by the Australian Research Council (FT100100779). PM is funded by the Swiss National Science Foundation (n°P300P3_154648).

503
504
505
506
507

508
509
510
511
512
513
514
515
516
517

Table 1. ANOVA-P values of soil parameters for main and interactive effects of treatments (FM: fertilizer manipulation; DR: drought manipulation), depth and time are reported (in bold when P<0.05). Individual graphs with mean and standard deviation are reported in Fig. 2 in supplementary information.

Factor	Soil parameter						
	Soil moisture	NH ₄ ⁺	NO ₃ ⁻	P	DON	Pom-C	Min-C
FM	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DR	0.0111	0.0441	0.0019	n.s.	n.s.	n.s.	n.s.
Depth	<.0001	<.0001	<.0001	<.0001	n.s.	<.0001	<.0001
Time	<.0001	<.0001	<.0001	n.s.	0.0025	<.0001	<.0001
FM * DR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
FM * Depth	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0426
DR * Depth	n.s.	n.s.	0.0406	n.s.	n.s.	n.s.	n.s.
Depth * Time	<.0001	0.0008	<.0001	n.s.	n.s.	0.01	n.s.
FM * DR * Depth	n.s.	n.s.	n.s.	n.s.	n.s.	0.0244	n.s.

*Interactive effects of time were almost never significant, therefore they are not reported.

518

519

Table 2. ANOVA-P values of main microbial groups for main and interactive effects of treatments (FM: fertilizer management; DR: drought), depth and time are reported (in bold when $P < 0.05$). Individual graphs with mean and standard deviation are reported in Fig. 2 in supplementary information.

Factor	Microbial groups					
	Total biomass	Fungi	Gram-positive	Gram-negative	Actinomycetes	F/B
FM	n.s	n.s	n.s	n.s	n.s	n.s
DR	n.s	n.s	n.s	n.s	n.s	n.s
Depth	<.0001	<.0001	<.0001	<.0001	<.0001	n.s
Time	n.s	n.s	n.s	0.0223	0.0202	n.s

*Interactive effects were almost never significant, therefore they are not reported.

521

522

523 References:

- 524 Ahrens, B., Braakhekke, M. C., Guggenberger, G., Schrumpf, M., and Reichstein, M. (2015).
 525 Contribution of sorption, DOC transport and microbial interactions to the 14C age of a
 526 soil organic carbon profile: Insights from a calibrated process model. *Soil Biology and*
 527 *Biochemistry* **88**, 390-402.
- 528 Anderson, T.-H., and Domsch, K. H. (1989). Ratios of microbial biomass carbon to total organic
 529 carbon in arable soils. *Soil Biology and Biochemistry* **21**, 471-479.
- 530 Bailey, V. L., Smith, J. L., and Bolton Jr, H. (2002). Fungal-to-bacterial ratios in soils
 531 investigated for enhanced C sequestration. *Soil Biology and Biochemistry* **34**, 997-1007.
- 532 Baldock, J. A., and Skjemstad, J. O. (2000). Role of the soil matrix and minerals in protecting
 533 natural organic materials against biological attack. *Organic Geochemistry* **31**, 697-710.
- 534 Barnard, R. L., Osborne, C. A., and Firestone, M. K. (2013). Responses of soil bacterial and
 535 fungal communities to extreme desiccation and rewetting. *ISME Journal* **7**, 2229-2241.
- 536 Bell, C., Acosta-Martinez, V., McIntyre, N., Cox, S., Tissue, D., and Zak, J. (2009). Linking
 537 Microbial Community Structure and Function to Seasonal Differences in Soil Moisture
 538 and Temperature in a Chihuahuan Desert Grassland. *Microbial Ecology* **58**, 827-842.
- 539 Bligh, E. G., and Dyer, W. J. (1959). A Rapid Method of Total Lipid Extraction and Purification.
 540 *Canadian Journal of Biochemistry and Physiology* **37**, 911-917.
- 541 Brockett, B. F. T., Prescott, C. E., and Grayston, S. J. (2012). Soil moisture is the major factor
 542 influencing microbial community structure and enzyme activities across seven
 543 biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry* **44**, 9-20.

- Bureau of Meteorology (2016).
http://www.bom.gov.au/climate/averages/tables/cw_067108.shtml.
- Canarini, A., and Dijkstra, F. A. (2015). Dry-rewetting cycles regulate wheat carbon rhizodeposition, stabilization and nitrogen cycling. *Soil Biology and Biochemistry* **81**, 195-203.
- Carrillo, Y., Dijkstra, F. A., LeCain, D., Morgan, J. A., Blumenthal, D., Waldron, S., and Pendall, E. (2014). Disentangling root responses to climate change in a semiarid grassland. *Oecologia* **175**, 699-711.
- Cleveland, C. C., Wieder, W. R., Reed, S. C., and Townsend, A. R. (2010). Experimental drought in a tropical rain forest increases soil carbon dioxide losses to the atmosphere. *Ecology* **91**(8), 2313-2323.
- Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., and Paul, E. (2013a). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Glob Chang Biol* **19**, 988-95.
- Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., and Paul, E. (2013b). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology* **19**, 988-995.
- Crowther, T. W., Sokol, N. W., Oldfield, E. E., Maynard, D. S., Thomas, S. M., and Bradford, M. A. (2015). Environmental stress response limits microbial necromass contributions to soil organic carbon. *Soil Biology and Biochemistry* **85**, 153-161.
- Dai, A. (2011). Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change* **2**, 45-65.
- de Vries, F. T., Hoffland, E., van Eekeren, N., Brussaard, L., and Bloem, J. (2006). Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biology and Biochemistry* **38**, 2092-2103.
- de Vries, F. T., Liiri, M. E., Bjornlund, L., Bowker, M. A., Christensen, S., Setälä, H. M., and Bardgett, R. D. (2012). Land use alters the resistance and resilience of soil food webs to drought. *Nature Climate Change* **2**, 276-280.
- de Vries, F. T., and Shade, A. (2013). Controls on soil microbial community stability under climate change. *Frontiers in Microbiology* **4**, 265.
- Diacono, M., and Montemurro, F. (2010). Long-term effects of organic amendments on soil fertility. A review. *Agron. Sustain. Dev.* **30**, 401-422.
- Feng, W., Plante, A. F., Aufdenkampe, A. K., and Six, J. (2014). Soil organic matter stability in organo-mineral complexes as a function of increasing C loading. *Soil Biology and Biochemistry* **69**, 398-405.
- Fierer, N., Schimel, J. P., and Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry* **35**, 167-176.
- Frostegård, A., and Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* **22**, 59-65.
- Frostegård, Å., Tunlid, A., and Bååth, E. (2011). Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry* **43**, 1621-1625.

- Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., and Richter, A. (2014). Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. *New Phytol* **201**, 916-27.
- Grace, J. B., Anderson, T. M., Olff, H., and Scheiner, S. M. (2010). On the specification of structural equation models for ecological systems. *Ecological Monographs* **80**, 67-87.
- Graciano, C., Guiamét, J. J., and Goya, J. F. (2005). Impact of nitrogen and phosphorus fertilization on drought responses in *Eucalyptus grandis* seedlings. *Forest Ecology and Management* **212**, 40-49.
- Gude, A., Kandeler, E., and Gleixner, G. (2012). Input related microbial carbon dynamic of soil organic matter in particle size fractions. *Soil Biology and Biochemistry* **47**, 209-219.
- Hamer, U., Unger, M., and Makeschin, F. (2007). Impact of air-drying and rewetting on PLFA profiles of soil microbial communities. *Journal of Plant Nutrition and Soil Science* **170**, 259-264.
- Handmer, J., Honda, Y., Kundzewicz, Z. W., Arnell, N., Benito, G., Hatfield, J., Mohamed, I. F., Peduzzi, P., Wu, S., Sherstyukov, B., Takahashi, K., Yan, Z., Vicuna, S., Suarez, A., Abdulla, A., Bouwer, L. M., Campbell, J., Hashizume, M., Hattermann, F., Heilmayr, R., Keating, A., Ladds, M., Mach, K. J., Mastrandrea, M. D., Mechler, R., Nobre, C., Sanghi, A., Screen, J., Smith, J., Velegrakis, A., Vergara, W., Waite, A. M., Westrich, J., Whittaker, J., Yunhe, Y., and Yamano, H. (2012). Changes in impacts of climate extremes: Human systems and ecosystems. In "Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation: Special Report of the Intergovernmental Panel on Climate Change", pp. 231-290.
- He, N., Chen, Q., Han, X., Yu, G., and Li, L. (2012). Warming and increased precipitation individually influence soil carbon sequestration of Inner Mongolian grasslands, China. *Agriculture, Ecosystems and Environment* **158**, 184-191.
- Hoover, D. L., and Rogers, B. M. (2016). Not all droughts are created equal: the impacts of interannual drought pattern and magnitude on grassland carbon cycling. *Glob Chang Biol* **22**, 1809-20.
- Huang, Y.-m., Michel, K., An, S.-s., and Zechmeister-Boltenstern, S. (2011). Changes in microbial-community structure with depth and time in a chronosequence of restored grassland soils on the Loess Plateau in northwest China. *Journal of Plant Nutrition and Soil Science* **174**, 765-774.
- Hueso, S., García, C., and Hernández, T. (2012). Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. *Soil Biology and Biochemistry* **50**, 167-173.
- Isbell, R. F. (2002). "The Australian Soil Classification," CSIRO Publishing, Melbourne.
- Jones, E., and Singh, B. (2014). Organo-mineral interactions in contrasting soils under natural vegetation. *Frontiers in Environmental Science* **2**.
- Kaisermann, A., Maron, P. A., Beaumelle, L., and Lata, J. C. (2015). Fungal communities are more sensitive indicators to non-extreme soil moisture variations than bacterial communities. *Applied Soil Ecology* **86**, 158-164.
- Kao-Kniffin, J., and Zhu, B. (2013). A Microbial Link between Elevated CO₂ and Methane Emissions that is Plant Species-Specific. *Microbial Ecology* **66**, 621-629.

- Kindler, R., Miltner, A., Richnow, H.-H., and Kästner, M. (2006). Fate of gram-negative bacterial biomass in soil—mineralization and contribution to SOM. *Soil Biology and Biochemistry* **38**, 2860-2870.
- Kindler, R., Miltner, A., Thullner, M., Richnow, H.-H., and Kästner, M. (2009). Fate of bacterial biomass derived fatty acids in soil and their contribution to soil organic matter. *Organic Geochemistry* **40**, 29-37.
- Kujur M., and K., P. A. (2014). PLFA Profiling of soil microbial community structure and diversity in different dry tropical ecosystems of Jharkhand. *Int.J.Curr.Microbiol.App.Sci* **3**, 556-575.
- Lamb, E. G., Kennedy, N., and Siciliano, S. D. (2010). Effects of plant species richness and evenness on soil microbial community diversity and function. *Plant and Soil* **338**, 483-495.
- Landesman, W. J., and Dighton, J. (2010). Response of soil microbial communities and the production of plant-available nitrogen to a two-year rainfall manipulation in the New Jersey Pinelands. *Soil Biology and Biochemistry* **42**, 1751-1758.
- Li, Y., Liu, Y., Wu, S., Niu, L., and Tian, Y. (2015). Microbial properties explain temporal variation in soil respiration in a grassland subjected to nitrogen addition. *Scientific Reports* **5**, 18496.
- Liang, C., Cheng, G., Wixon, D., and Balser, T. (2011). An Absorbing Markov Chain approach to understanding the microbial role in soil carbon stabilization. *Biogeochemistry* **106**, 303-309.
- Lindahl, V., Frostegård, Å., Bakken, L., and Bååth, E. (1997). Phospholipid fatty acid composition of size fractionated indigenous soil bacteria. *Soil Biology and Biochemistry* **29**, 1565-1569.
- Liu, L., Gundersen, P., Zhang, T., and Mo, J. (2012). Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biology and Biochemistry* **44**, 31-38.
- Liu, Q., Qiao, N., Xu, X., Xin, X., Han, J. Y., Tian, Y., Ouyang, H., and Kuzyakov, Y. (2016). Nitrogen acquisition by plants and microorganisms in a temperate grassland. *Scientific Reports* **6**, 22642.
- Maestre, F. T., Delgado-Baquerizo, M., Jeffries, T. C., Eldridge, D. J., Ochoa, V., Gozalo, B., Quero, J. L., García-Gómez, M., Gallardo, A., Ulrich, W., Bowker, M. A., Arredondo, T., Barraza-Zepeda, C., Bran, D., Florentino, A., Gaitán, J., Gutiérrez, J. R., Huber-Sannwald, E., Jankju, M., Mau, R. L., Miriti, M., Naseri, K., Ospina, A., Stavi, I., Wang, D., Woods, N. N., Yuan, X., Zaady, E., and Singh, B. K. (2015). Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences* **112**, 15684-15689.
- Manzoni, S., Schimel, J. P., and Porporato, A. (2012). Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology* **93**, 930-938.
- Mariotte, P., Robroek, B. J. M., Jassey, V. E. J., and Buttler, A. (2015). Subordinate plants mitigate drought effects on soil ecosystem processes by stimulating fungi. *Functional Ecology* **29**, 1578-1586.
- McHugh, T. A., and Schwartz, E. (2014). Changes in plant community composition and reduced precipitation have limited effects on the structure of soil bacterial and fungal communities present in a semiarid grassland. *Plant and Soil* **388**, 175-186.

- Merino, C., Nannipieri, P., and Matus, F. (2015). Soil carbon controlled by plant, microorganism and mineralogy interactions. *Journal of Soil Science and Plant Nutrition* **15**, 321-332.
- Milcu, A., Allan, E., Roscher, C., Jenkins, T., Meyer, S. T., Flynn, D., Bessler, H., Buscot, F., Engels, C., Gubsch, M., König, S., Lipowsky, A., Loranger, J., Renker, C., Scherber, C., Schmid, B., Thébault, E., Wubet, T., Weisser, W. W., Scheu, S., and Eisenhauer, N. (2013). Functionally and phylogenetically diverse plant communities key to soil biota. *Ecology* **94**, 1878-1885.
- Min, S.-K., Zhang, X., Zwiers, F. W., and Hegerl, G. C. (2011). Human contribution to more-intense precipitation extremes. *Nature* **470**, 378-381.
- Muthén, L. K., and Muthén, B. O. (1998-2010). Mplus User's Guide.
- Nelson, A. G., Quideau, S., Frick, B., Niziol, D., Clapperton, J., and Spaner, D. (2011). Spring wheat genotypes differentially alter soil microbial communities and wheat breadmaking quality in organic and conventional systems. *Canadian Journal of Plant Science* **91**, 485-495.
- Nie, M., Pendall, E., Bell, C., Gasch, C. K., Raut, S., Tamang, S., and Wallenstein, M. D. (2013). Positive climate feedbacks of soil microbial communities in a semi-arid grassland. *Ecology Letters* **16**, 234-241.
- Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H. (2015). Package 'vegan'. *Community ecology package, version, 2.2-1*.
- Olsen, S. R., and Sommers, L. E. (1982). Phosphorus. In "Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties" (A. L. Pace, R. H. Miller and D. R. Keeney, eds.), pp. 403-430. American Society of Agronomy, Inc., Madison, WI.
- Olsson, P. A. (1999). Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology* **29**, 303-310.
- Pausch, J., Kramer, S., Scharroba, A., Scheunemann, N., Butenschön, O., Kandeler, E., Marhan, S., Riederer, M., Scheu, S., Kuzyakov, Y., and Ruess, L. (2016). Small but active – pool size does not matter for carbon incorporation in below-ground food webs. *Functional Ecology* **30**, 479-489.
- R Core Team (2013). R: A language and environment for statistical computing. . R Foundation for Statistical Computing, Vienna, Austria.
- Rousk, J., Smith, A. R., and Jones, D. L. (2013). Investigating the long-term legacy of drought and warming on the soil microbial community across five European shrubland ecosystems. *Global Change Biology* **19**, 3872-3884.
- Ruess, L., and Chamberlain, P. M. (2010). The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biology and Biochemistry* **42**, 1898-1910.
- Sahs, W. W., and Lesoing, G. (1985). Crop rotations and manure versus agricultural chemicals in dryland grain production. *Journal of Soil and Water Conservation* **40**, 511-516.
- Scharlemann, J. P. W., Tanner, E. V. J., Hiederer, R., and Kapos, V. (2014). Global soil carbon: understanding and managing the largest terrestrial carbon pool. *Carbon Management* **5**, 81-91.
- Scheunemann, N. (2015). Carbon flow in belowground food webs assessed by isotope tracers. Doctoral thesis, Georg-August-Universität Göttingen.

- Schimel, J., Balser, T. C., and Wallenstein, M. (2007). Microbial stress-response physiology and its implications for ecosystem function. *Ecology* **88**, 1386-1394.
- Schimel, J. P., and Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Front Microbiol* **3**, 348.
- Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D. A., Nannipieri, P., Rasse, D. P., Weiner, S., and Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature* **478**, 49-56.
- Schurig, C., Smittenberg, R. H., Berger, J., Kraft, F., Woche, S. K., Goebel, M., Heipieper, H. J., Miltner, A., and Kaestner, M. (2013). Microbial cell-envelope fragments and the formation of soil organic matter: A case study from a glacier forefield. *Biogeochemistry* **113**, 595-612.
- Six, J., Frey, S. D., Thiet, R. K., and Batten, K. M. (2006). Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. *Soil Science Society of America Journal* **70**, 555.
- Solomon, D., Lehmann, J., Harden, J., Wang, J., Kinyangi, J., Heymann, K., Karunakaran, C., Lu, Y., Wirick, S., and Jacobsen, C. (2012). Micro- and nano-environments of carbon sequestration: Multi-element STXM-NEXAFS spectromicroscopy assessment of microbial carbon and mineral associations. *Chemical Geology* **329**, 53-73.
- Spohn, M., Klaus, K., Wanek, W., and Richter, A. (2016). Microbial carbon use efficiency and biomass turnover times depending on soil depth – Implications for carbon cycling. *Soil Biology and Biochemistry* **96**, 74-81.
- Tian, X. F., Hu, H. W., Ding, Q., Song, M. H., Xu, X. L., Zheng, Y., and Guo, L. D. (2014). Influence of nitrogen fertilization on soil ammonia oxidizer and denitrifier abundance, microbial biomass, and enzyme activities in an alpine meadow. *Biology and Fertility of Soils* **50**, 703-713.
- Trenberth, K. E., Dai, A., van der Schrier, G., Jones, P. D., Barichivich, J., Briffa, K. R., and Sheffield, J. (2014). Global warming and changes in drought. *Nature Clim. Change* **4**, 17-22.
- Treseder, K. K. (2008). Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol Lett* **11**, 1111-20.
- Vries, F., Liiri, M., Bjørnlund, L., Setälä, H., Christensen, S., and Bardgett, R. (2012). Legacy effects of drought on plant growth and the soil food web. *Oecologia* **170**, 821-833.
- Wallenstein, M. D., McNulty, S., Fernandez, I. J., Boggs, J., and Schlesinger, W. H. (2006). Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management* **222**, 459-468.
- Yang, K., and Zhu, J. (2015). The effects of N and P additions on soil microbial properties in paired stands of temperate secondary forests and adjacent larch plantations in Northeast China. *Soil Biology and Biochemistry* **90**, 80-86.
- You, Y., Wang, J., Huang, X., Tang, Z., Liu, S., and Sun, O. J. (2014). Relating microbial community structure to functioning in forest soil organic carbon transformation and turnover. *Ecology and Evolution* **4**, 633-647.
- Zelles, L. (1997). Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* **35**, 275-294.

- Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils* **29**, 111-129.
- Zhang, L., Wylie, B. K., Ji, L., Gilmanov, T. G., and Tieszen, L. L. (2010). Climate-Driven Interannual Variability in Net Ecosystem Exchange in the Northern Great Plains Grasslands. *Rangeland Ecology & Management* **63**, 40-50.
- Zhang, L., Wylie, B. K., Ji, L., Gilmanov, T. G., Tieszen, L. L., and Howard, D. M. (2011). Upscaling carbon fluxes over the Great Plains grasslands: Sinks and sources. *Journal of Geophysical Research* **116**.
- Zhang, N., Liu, W., Yang, H., Yu, X., Gutknecht, J. L. M., Zhang, Z., Wan, S., and Ma, K. (2013). Soil microbial responses to warming and increased precipitation and their implications for ecosystem C cycling. *Oecologia* **173**, 1125-1142.
- Zhang, N., Wan, S., Guo, J., Han, G., Gutknecht, J., Schmid, B., Yu, L., Liu, W., Bi, J., Wang, Z., and Ma, K. (2015). Precipitation modifies the effects of warming and nitrogen addition on soil microbial communities in northern Chinese grasslands. *Soil Biology and Biochemistry* **89**, 12-23.

Figures legends:

Fig. 1: Volumetric soil moisture content (v/v) and precipitation (mm) from April 2014 to January 2015. Soil moisture content is shown for the four treatments and averaged from first and last block (n = 2, Amb-MN: ambient precipitation and mineral fertilizer addition; Amb-CP: ambient precipitation and compost addition; Red-MN: reduced precipitation and mineral fertilizer addition; Red-CP: reduced precipitation and compost addition).

Fig. 2. Mean values of soil moisture, NH_4^+ , NO_3^- , available P, Pom-C and Min-C for the four treatments at the three sampling dates. Graphs from a) to f) represent surface soil layer (0-5 cm), while from g) to l) deeper soil layer (5-15 cm). Error bars represent standard error of the means.

Fig. 3: Abundance of main decomposer groups in treatment plots as determined by PLFA. Microbial groups are averaged by time. Error bars are standard error of the total mean. Bars are

grouped by treatment (Amb-MN: ambient precipitation and mineral fertilizer addition; Amb-CP: ambient precipitation and compost addition; Red-MN: reduced precipitation and mineral fertilizer addition; Red-CP: reduced precipitation and compost addition) and divided in the two depths (surface: 0-5 cm; deep: 5-15 cm). Asterisks represent significance difference between the two depths as tested by ANOVA in Table 2.

Fig. 4: Path analysis model for the effect of Pom-C, soil moisture and NO_3^- on microbial biomass (Tot PLFA) and Fungi/Bacteria (F/B) ratio and their effects on Min-C in surface (a) and deep soil (b). Arrows with different width represent different standardized effect sizes as shown in the legend. Significant values are indicated by * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$). R^2 values are indicated for the dependent variables. Double arrowed lines represent correlation without establishment of casualty. Our overall model fit was satisfactory (Surface: $\chi^2 = 6.6$, $P = 0.16$, TLI = 0.90, CFI = 0.90, RMSEA = 0.098; Deep: $\chi^2 = 6.4$, $P = 0.06$, TLI = 0.90, CFI = 0.97, RMSEA = 0.056).

Fig. 5: Path analysis model for the effect of Pom-C, soil moisture and NO_3^- on main decomposer group and their effects on Min-C in surface (top graph) and deep soil (bottom graph). Arrows with different width represent different standardized effect sizes as shown in the legend. Significant values are indicated by * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$). R^2 values are indicated for the dependent variables. Double arrowed lines represent correlation without establishment of casualty. Our overall model fit was satisfactory (Surface: $\chi^2 = 3.1$, $P = 0.38$, TLI = 0.99, CFI = 0.99, RMSEA = 0.025; Deep: $\chi^2 = 10.2$, $P = 0.18$, TLI = 0.98, CFI = 0.99, RMSEA = 0.097).

Figure

Figure 1. Canarini et al.

Figure 2. Canarini et al.

Figure 3. Canarini et al.

a)

b)

Figure 4. Canarini et al.

Figure 5. Canarini et al.