Short-term responses and resistance of soil microbial community structure to elevated CO2 and N addition in grassland mesocosms

Marie Simonin, Naoise Nunan, Juliette Bloor, Valérie Pouteau, Audrey Niboyet

FEMS Microbiology Letters, 364, 2017, fnx077

doi: 10.1093/femsle/fnx077
Advance Access Publication Date: 18 April 2017
Research Letter

RESEARCH LETTER – Environmental Microbiology

Short-term responses and resistance of soil microbial community structure to elevated CO_2 and N addition in grassland mesocosms

Marie Simonin[1,*,†], Naoise Nunan[1], Juliette M. G. Bloor[2], Valérie Pouteau[1] and Audrey Niboyet[1]

[1]Institute of Ecology and Environmental Sciences–Paris, UMR 7618 Université Pierre et Marie Curie/CNRS/AgroParisTech, AgroParisTech, 78850 Thiverval-Grignon, France and [2]INRA, UR0874 Grassland Ecosystem Research Unit, F-63039 Clermont-Ferrand, France

*Corresponding author: Institute of Ecology and Environmental Sciences–Paris, UMR 7618 Université Pierre et Marie Curie/CNRS/AgroParisTech, AgroParisTech, 78850 Thiverval-Grignon, France. Tel: (919) 684-6556; E-mail: simonin.marie@gmail.com
†Present address: Department of Biology, Duke University, Durham, NC, USA.
One sentence summary: Elevated CO_2 alone or in combination with N addition does not affect soil microbial community structure in grassland mesocosms.
Editor: Juan Imperial

ABSTRACT

Nitrogen (N) addition is known to affect soil microbial communities, but the interactive effects of N addition with other drivers of global change remain unclear. The impacts of multiple global changes on the structure of microbial communities may be mediated by specific microbial groups with different life-history strategies. Here, we investigated the combined effects of elevated CO_2 and N addition on soil microbial communities using PLFA profiling in a short-term grassland mesocosm experiment. We also examined the linkages between the relative abundance of r- and K-strategist microorganisms and resistance of the microbial community structure to experimental treatments. N addition had a significant effect on microbial community structure, likely driven by concurrent increases in plant biomass and in soil labile C and N. In contrast, microbial community structure did not change under elevated CO_2 or show significant $CO_2 \times N$ interactions. Resistance of soil microbial community structure decreased with increasing fungal/bacterial ratio, but showed a positive relationship with the Gram-positive/Gram-negative bacterial ratio. Our findings suggest that the Gram-positive/Gram-negative bacteria ratio may be a useful indicator of microbial community resistance and that K-strategist abundance may play a role in the short-term stability of microbial communities under global change.

Keywords: global change; grassland; fungal/bacterial ratio; PLFA

INTRODUCTION

Growing awareness of the key role of soil biodiversity for the maintenance of ecosystem services has led to considerable interest in the impacts of global change on belowground diversity in recent years (Bardgett and van der Putten 2014). Elevated CO_2 and nitrogen (N) fertilisation or deposition are two main components of global change that may have significant consequences for microbial community structure and microbial-mediated processes. Empirical evidence suggests that N additions increase the fungal/bacterial ratio in grasslands in the

Received: 13 November 2016; **Accepted:** 17 April 2017

short-term (Bardgett *et al.* 1999), but decrease this ratio in the longer term (>1 year; Bardgett and McAlister 1999; Bradley, Drijber and Knops 2006; Clegg 2006; Denef *et al.* 2009; Rousk, Brookes and Bååth 2011). In contrast, previous grassland studies have reported inconsistent responses of soil microbial community structure to elevated carbon dioxide (CO_2): elevated CO_2 had either no effect on microbial community structure (Ebersberger *et al.* 2004; Gutknecht, Field and Balser 2012) or led to significant increases in fungal biomarkers and fungal/bacterial ratio (Kandeler *et al.* 2008; Guenet *et al.* 2012). In theory, increases in the fungal/bacterial ratio driven by decreased soil N availability under elevated CO_2 could be offset by N addition, especially in N-limited ecosystems (Lagomarsino *et al.* 2007). However, microbial community responses to simultaneous increases in N and CO_2 have attracted little attention to date (Gutknecht, Field and Balser 2012).

The response of soil microbial communities to global changes may be mediated by the plant community via changes in plant biomass or plant-induced changes in soil resource availability, such as labile C or mineral N contents (Bardgett, Freeman and Ostle 2008; Gutknecht, Field and Balser 2012; Drigo *et al.* 2013; Philippot *et al.* 2013). Moreover, resistance of the microbial community structure (i.e. the degree to which microbial composition remains unchanged in the face of a disturbance, Allison and Martiny 2008) to disturbances (i.e. events that alter directly or indirectly a community, usually through effects on the environment of the community) may depend on the relative abundance and contribution of specific functional groups and their life-history strategies (Schimel, Balser and Wallenstein 2007; Wallenstein and Hall 2011). For example, r-strategists exhibit high growth rates and consume soil labile carbon, while K-strategists present slower growth rates and are likely to outcompete r-strategists in conditions of low nutrient availability due to their higher substrate affinities (Fierer, Bradford and Jackson 2007). Numerous studies suggest that K-strategist microorganisms are more resistant to global change induced disturbances (de Vries and Shade 2013; Bischoff *et al.* 2016; Villa *et al.* 2016; Zhang *et al.* 2016). The fungal/bacterial ratio and Gram-positive/Gram-negative bacteria ratios have been proposed as proxies of the prevalence of K-strategists in the microbial community and are thus expected to be positively related to microbial community resistance (de Vries and Shade 2013).

In this experiment, we investigate the combined effects of elevated CO_2 and N supply on soil microbial community structure in grassland mesocosms planted with *Dactylis glomerata*. Previous work published on this mesocosm experiment has shown that soil microbial processes related to C and N cycling were altered by the combined effects of elevated CO_2 and N addition (Niboyet *et al.* 2010), and that the abundance of nitrifiers was altered by experimental treatments (Simonin *et al.* 2015). We examine how soil PLFA profiles are affected by elevated CO_2 and N addition, and analyse the links between the relative abundance of r- and K-strategists and the resistance of the soil microbial community structure to these disturbances.

MATERIALS AND METHODS

Experimental design

The mesocosm experiment was performed in growth chambers at the Université Paris Sud (Orsay, France) and comprised two treatments in a factorial design: atmospheric CO_2 (ambient 381 $\pm$ 6 μmol mol^{-1} vs elevated 645 $\pm$ 9 μmol mol^{-1}) and N addition (no N addition vs +10 g N-NH_4NO_3 m^{-2}). The elevated CO_2 concentration corresponds to the intermediary IPCC projections at the horizon 2050 (Stocker 2014), whereas the N addition (equivalent to 100 kg N ha^{-1}) is in line with local fertiliser practices (Bloor, Barthes and Leadley 2008). Each mesocosm consisted of a PVC pot (15 $\times$ 20 $\times$ 50 cm) filled with soil from a nearby grassland (pH = 8.5, 2.46 g C kg^{-1}, organic matter content 4.26 g kg^{-1}, 0.23 g N kg^{-1}, cation exchange capacity 1.81 cmol kg^{-1}). Mesocosms were sown with *Dactylis glomerata* and two mesocosms were placed in each of 12 growth chambers within a large glasshouse. CO_2 was manipulated at the chamber level whereas N was manipulated at the mesocosm level; each growth chamber contained one replicate mesocosm of each N treatment such that each CO_2 $\times$ N treatment combination was replicated six times (total of 24 mesocosms). The treatments were initiated 1 month after sowing, when the *D. glomerata* seedlings had fully emerged (Niboyet *et al.* 2010), and the mesocosms were harvested after 10 weeks under treatments. The aboveground (shoot) and belowground (root) plant biomass were collected, washed and oven-dried (60°C, 72 h) prior to weighing to determine dry mass. Samples from the top soil (0–10 cm) were collected at the final harvest and sieved at 2 mm, and then immediately used for measurements of soil water content, soil respiration and gross N mineralisation rates (see Niboyet *et al.* 2010 for additional details). In addition, soil samples were stored at –20°C for PLFA extraction.

Analysis of the soil microbial community structure by PLFA profiles

A solution of chloroform (15 mL), methanol (30 mL) and citrate buffer (12 mL) was used to extract the lipids from 10 g of freeze-dried soil (Frostegård, Tunlid and Bååth 1993). After extraction and derivatisation, the fatty acid methyl esters (FAME) were characterised based on a standard bacterial acid methyl ester (BAME) ranging from 11:0 to 20:0. We used an Agilent 6890 gas chromatograph equipped with a Flame Ionisation Detector (GC-FID) and a SGE-BPX5 column (65 m $\times$ 320 μm $\times$ 0.25 μm) to quantify the FAME extracted from the soil samples based on their retention time and mass spectral comparison. Standard fatty acid nomenclature was used following Frostegård, Tunlid and Bååth (1993). Seventeen lipids were identified and for each lipid, the relative area of the corresponding peak was calculated. The assignments of PLFA regarded as bacterial biomarkers, fungal biomarker, Gram-negative bacterial biomarkers and Gram-positive bacterial biomarkers were performed as described by O'Leary and Wilkinson (1988), Frostegård, Tunlid and Bååth (1993), Frostegård and Bååth (1996) and Zelles (1997, 1999). These biomarkers were used to compute the relative abundance of bacteria, fungi and the Gram-positive/Gram-negative and fungal/bacterial ratios.

Statistical analysis of PLFA profiles

The experiment was represented as a fully factorial split-plot design: the whole-plot factor is the CO_2 treatment and the N treatment is the split-plot factor.

The PLFA patterns of the soil samples were analysed in R (R Core Team 2015) by non-metric multidimensional scaling (NMDS) with the 'Vegan: Community Ecology Package' (Oksanen *et al.* 2007). Bray-Curtis similarities were used to build a distance matrix for the NMDS. Permutational multivariate analysis of variance (PERMANOVA) was then used to test whether treatments altered PLFA patterns using the *adonis* function (999 permutations) of the Vegan package.

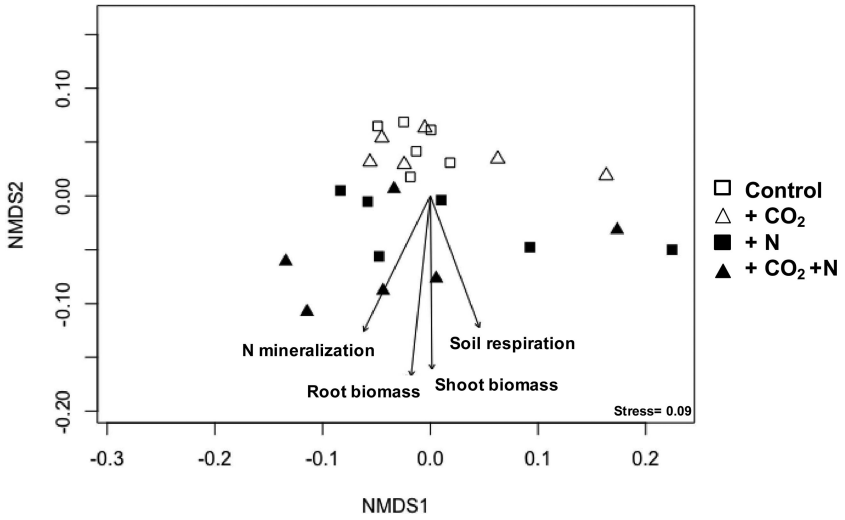

Figure 1. NMDS of the PLFA pattern (17 lipids) in *D. glomerata* mesocosms in the four treatments (n = 6 in each treatment). Root and shoot biomass, soil respiration and gross N mineralisation rates are represented as vectors as they were found to be significant explanatory variables ($P < 0.05$).

The interactive effects of elevated CO_2 and N supply on the relative abundance of bacteria and fungi, and on the ratios between the relative abundance of fungal/bacterial and Gram positive/Gram negative biomarkers were assessed using PROC MIXED in SAS 9.3 (SAS Institute, Cary, NC, USA).

Correlations between NMDS scores and potential drivers of microbial community structure (plant biomass, soil respiration, gross N mineralisation and soil water content; Niboyet *et al.* 2010) were assessed with the *envfit* function and post hoc permutation tests (n = 999). Soil respiration was used as an indicator of soil labile carbon, and gross N mineralisation rates as a proxy of soil N availability.

Resistance of the soil microbial community structure

To measure the resistance of the soil microbial community structure to the disturbances, we calculated Bray-Curtis similarities in microbial communities in disturbed treatments (CO_2, N or CO_2 + N) relative to the control treatment given by the PLFA profiles (de Vries and Shade 2013) using the *vegdist* function (Vegan in R). A similarity of 1 indicates maximum resistance, i.e. no effect of disturbance on microbial community structure, while a similarity close to 0 indicates very low resistance. Correlations between the resistance index and Gram-positive/Gram-negative ratio or fungal/bacterial ratio were examined using Proc CORR in SAS 9.3.

RESULTS

Elevated CO_2 and N addition effects on soil microbial community structure

Multivariate analysis (NMDS) indicated that N addition significantly altered the microbial community structure (PERMANOVA: $P = 0.003$) (Fig. 1). In contrast, elevated CO_2 had no significant effect ($P = 0.86$) and no significant $CO_2 \times N$ interactions were detected ($P = 0.38$). The addition of N significantly increased the relative abundance of fungi ($P < 0.05$, +78%, Fig. 2A) and the fungal/bacterial ratio ($P < 0.001$, +95%, Fig. 2B), while CO_2, alone or in combination with the N treatment, had no effect on these variables (Fig. 2). The bacterial relative abundance (Fig. 2A), the Gram+ and Gram– relative abundance (data not shown), and the ratio of Gram-positive/Gram-negative bacterial biomarkers (Fig. 2B) were not affected by any of the treatments.

Relationships between PLFA profiles and plant and soil variables

The correlations with the NMDS axis 2 indicate that the effect of the N treatment on the soil microbial community structure seemed to be mainly related to a gradient in root and shoot biomass and in soil respiration (a proxy of soil labile C availability) and gross N mineralisation rates (a proxy of soil mineral N availability) (Fig. 1, Table S2, Supporting Information). In this experiment, N addition had a positive effect on root and shoot biomass ($P < 0.001$ in both cases), especially under elevated CO_2 ($P = 0.02$ and $P = 0.003$, respectively, Table S1, Supporting Information). Elevated CO_2 and N addition had positive effects on soil respiration ($P = 0.03$ and $P < 0.001$, respectively, Table S1, Supporting Information). N addition also increased gross N mineralisation rates when combined with elevated CO_2 ($P = 0.03$, Table S1, Supporting Information).

Resistance of the soil microbial community structure to the environmental change

The resistance of the PLFA profiles to the disturbances (i.e. to the imposed increases in CO_2, N or in both CO_2 and N) decreased with the fungal/bacterial ratio (Fig. 3A) but increased with the ratio of Gram-positive/Gram-negative bacteria (Fig. 3B). These correlations also tended to be observed when considering each disturbance separately (Table S3, Supporting Information).

DISCUSSION

Increases in fungal biomass after the addition of N have been frequently observed in short-term studies, i.e. in studies conducted over several months (Bardgett *et al.* 1999; Gallo *et al.* 2004), where the progressive inhibition of growth or the decrease of mycorrhizal fungal biomass by N has not already occurred (Bardgett and McAlister 1999; Gallo *et al.* 2004; Treseder 2008). Our results confirm an N-induced increase in both the relative abundance of fungi and the fungal/bacterial ratio. Stimulation

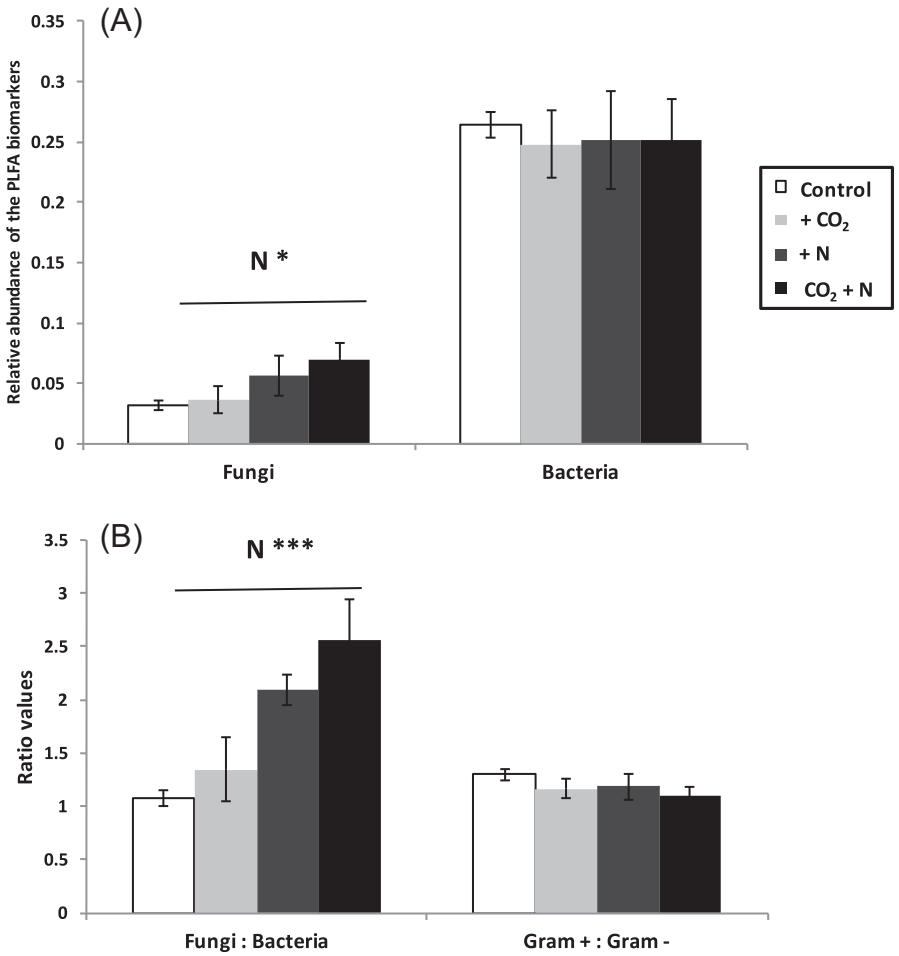

Figure 2. Relative abundance of the bacterial and fungal biomarkers (**A**) and ratios of the PLFA relative abundance of fungi/bacteria and Gram-positive/Gram-negative bacteria (**B**) in the four treatments. The means and standard errors (n = 6) are presented. Significant effects are indicated (*$P < 0.05$, *** $P < 0.001$).

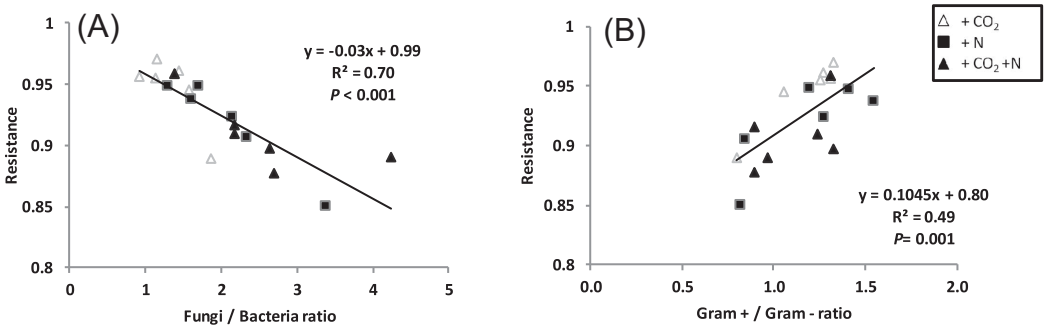

Figure 3. Pearson's correlations between ratios of the PLFA relative abundance of fungi/bacteria (**A**) and Gram-positive/Gram-negative bacteria (**B**) and the resistance of the soil microbial community structure to the treatments (elevated CO_2, N addition and elevated CO_2 combined with N addition). The linear regressions are drawn and the associated equations, R-square and *P* values are given.

of root and shoot biomass by the supply of N likely resulted in an increase in C inputs via rhizodeposition (Niboyet *et al.* 2010), as suggested by the large increase in soil respiration with N addition. The resulting higher C availability seems to have benefited fast-growing fungi, specialised in the metabolism of large quantities of labile C substrates, and not bacteria (Chigineva, Aleksandrova and Tiunov 2009; de Graaff *et al.* 2010, de Vries and Caruso 2016). In our experiment, elevated CO_2 did not alter the microbial community structure, in agreement with other short- and long-term studies (Zak *et al.* 1996; Ebersberger *et al.* 2004; Gutknecht, Field and Balser 2012). The absence of CO_2 effects and of interactive effects with N addition might reflect the lack of negative-CO_2 effects on soil mineral N availability in this study.

Microbial functional traits, and especially the relative abundance in a community of r- and K-strategists, may be useful indicators of the response of the soil microbial community structure to stressors, such as global environmental changes. In our study, the Gram-positive/Gram-negative ratio was positively correlated with the resistance of the microbial community

structure; assuming that the Gram positive/Gram negative ratio is a proxy of the presence of K-strategists, these results are consistent with the idea that K-strategists promote microbial community resistance (de Vries and Shade 2013). In our study, the Gram-positive/Gram-negative ratio only explained 49% of the variance of the resistance to the treatments, which is probably related to the limited effects of the treatments on the Gram-positive/Gram-negative ratio.

Surprisingly, the fungal/bacterial ratio, which is potentially also an indicator of the proportion of K-strategists, was negatively correlated with the resistance of the microbial community structure. One possible explanation is that the fungal community comprises both r- and K-strategists, and that in this short-term experiment, the fungal community was dominated by fast-growing fungi (i.e. r-strategists) that experienced drastic changes in composition and abundance. Our findings thus suggest that the Gram-positive/Gram-negative bacteria ratio may be a useful indicator of microbial community resistance to elevated CO_2 and N addition and that a higher relative abundance of K-strategist may promote the stability of the microbial community under global change. Other functional and taxonomic traits of soil microbial communities such as genome size, 16S rDNA gene copy number or mean generation time (Fierer, Bradford and Jackson 2007; Leff *et al.* 2015) should also be considered to confirm the role of K-strategists for the resistance of the soil microbial community structure to disturbances under future environmental changes.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

ACKNOWLEDGEMENTS

The authors thank the two anonymous reviewers for their constructive comments. The authors thank the persons involved in conducting the mesocosm experiment and in particular Annick Ambroise, Laure Barthes, Sandrine Fontaine and Paul Leadley from the Laboratoire Ecologie Systématique, Evolution (UMR 8079).

FUNDING

This study was funded by AgroParisTech support of the Institute of Ecology and Environmental Sciences – Paris (UMR 7618).

Conflict of interest. None declared.

REFERENCES

Allison SD, Martiny JBH. Resistance, resilience, and redundancy in microbial communities. *PNAS* 2008;**105**:11512–9.

Bardgett RD, Freeman C, Ostle NJ. Microbial contributions to climate change through carbon cycle feedbacks. *ISME J* 2008;**2**:805–14.

Bardgett RD, Mawdsley JL, Edwards S *et al.* Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Funct Ecol* 1999;**13**:650–60.

Bardgett RD, McAlister E. The measurement of soil fungal: bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. *Biol Fertil Soils* 1999;**29**:282–90.

Bardgett RD, van der Putten WH. Belowground biodiversity and ecosystem functioning. *Nature* 2014;**515**:505–11.

Bischoff N, Mikutta R, Shibistova O *et al.* Land-use change under different climatic conditions: Consequences for organic matter and microbial communities in Siberian steppe soils. *Agr Ecosyst Environ* 2016;**235**:253–64.

Bloor J, Barthes L, Leadley P. Effects of elevated CO_2 and N on tree–grass interactions: an experimental test using Fraxinus excelsior and Dactylis glomerata. *Funct Ecol* 2008;**22**:537–46.

Bradley K, Drijber RA, Knops J. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. *Soil Biol Biochem* 2006;**38**:1583–95.

Chigineva NI, Aleksandrova AV, Tiunov AV. The addition of labile carbon alters litter fungal communities and decreases litter decomposition rates. *Appl Soil Ecol* 2009;**42**:264–70.

Clegg CD. Impact of cattle grazing and inorganic fertiliser additions to managed grasslands on the microbial community composition of soils. *Appl Soil Ecol* 2006;**31**:73–82.

de Graaff M-A, Classen AT, Castro HF *et al.* Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. *New Phytol.* 2010;**188**:1055–64.

Denef K, Roobroeck D, Wadu MCM *et al.* Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. *Soil Biol Biochem* 2009;**41**:144–53.

de Vries FT, Caruso T. Eating from the same plate? Revisiting the role of labile carbon inputs in the soil food web. *Soil Biol Biochem* 2016;**102**:4–9.

de Vries FT, Shade A. Controls on soil microbial community stability under climate change. *Front Microbiol* 2013;**4**:265.

Drigo B, Kowalchuk GA, Knapp BA *et al.* Impacts of 3 years of elevated atmospheric CO_2 on rhizosphere carbon flow and microbial community dynamics. *Glob Change Biol* 2013;**19**:621–36.

Ebersberger D, Wermbter N, Niklaus PA *et al.* Effects of long term CO_2 enrichment on microbial community structure in calcareous grassland. *Plant Soil* 2004;**264**:313–23.

Fierer N, Bradford MA, Jackson RB. Toward an ecological classification of soil bacteria. *Ecology* 2007;**88**:1354–64.

Frostegård A, Bååth E. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol Fertil Soil* 1996;**22**:59–65.

Frostegård A, Tunlid A, Bååth E. Phospholipidfatty acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Appl Environ Microb* 1993;**59**:3605–17.

Gallo M, Amonette R, Lauber C *et al.* Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microb Ecol* 2004;**48**:218–29.

Guenet B, Lenhart K, Leloup J *et al.* The impact of long-term CO_2 enrichment and moisture levels on soil microbial community structure and enzyme activities. *Geoderma* 2012;**170**:331–6.

Gutknecht JLM, Field CB, Balser TC. Microbial communities and their responses to simulated global change fluctuate greatly over multiple years. *Glob Change Biol* 2012;**18**:2256–69.

Kandeler E, Mosier A, Morgan J *et al.* Transient elevation of CO_2 modifies the microbial community composition in a semi-arid grassland. *Soil Biol Biochem* 2008;**40**:162–71.

Lagomarsino A, Knapp B, Moscatelli M *et al.* Structural and functional diversity of soil microbes is affected by elevated [CO_2] and N addition in a poplar plantation. *J Soils Sediments* 2007;**7**:399–405.

Leff JW, Jones SE, Prober SM *et al*. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *P Natl Acad Sci USA* 2015;**112**:10967–72.

Niboyet A, Barthes L, Hungate B *et al*. Responses of soil nitrogen cycling to the interactive effects of elevated CO_2 and inorganic N supply. *Plant Soil* 2010;**327**:35–47.

Oksanen J, Kindt R, Legendre P *et al*. *The Vegan: Community Ecology Package*, 2007. R package version 2.4-2. https://CRAN.R-project.org/package=vegan.

O'Leary WM, Wilkinson SG. Gram-positive bacteria. In: Ratledge C, Wilkinson SG. (eds.), *Microbial Lipids*. London: Academic Press. 1988, 117–2002.

Philippot L, Raaijmakers JM, Lemanceau P *et al*. Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 2013;**11**:789–99.

R Core Team. R Foundation for Statistical Computing, Vienna, Austria. *R: A Language and Environment for Statistical Computing*. https://www.R-project.org/, New York, NY: Cambridge University Press. 2015.

Rousk J, Brookes PC, Bååth E. Fungal and bacterial growth responses to N fertilization and pH in the 150-year "Park Grass" UK grassland experiment. *FEMS Microbiol Ecol* 2011; **76**:89–99.

Schimel J, Balser T, Wallenstein M. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 2007;**88**:1386–94.

Simonin M, Roux XL, Poly F *et al*. Coupling between and among ammonia oxidizers and Nitrite oxidizers in grassland mesocosms submitted to Elevated CO_2 and nitrogen supply. *Microb Ecol* 2015;**70**:809–18.

Stocker TF. Climate change 2013: the physical science basis. *Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, 2014.

Treseder KK. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol Lett* 2008;**11**:1111–20.

Villa F, Stewart PS, Klapper I *et al*. Subaerial biofilms on outdoor stone monuments: changing the perspective toward an ecological framework. *Bioscience* 2016; **66**:285–94.

Wallenstein MD, Hall EK. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry* 2011;**109**: 35–47.

Zak DR, Ringelberg DB, Pregitzer KS *et al*. Soil microbial communities beneath Populus grandidentata grown under elevated atmospheric CO_2. *Ecol Appl* 1996; **6**:257–62.

Zelles L. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol Fertil Soils* 1999;**29**:111–29.

Zelles L. Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* 1997;**35**:275–94.

Zhang K, Shi Y, Jing X *et al*. Effects of short-term warming and altered precipitation on soil microbial communities in alpine grassland of the tibetan plateau. *Front Microbiol* 2016;**7**:1032.