Short communication

Interactive effects of precipitation and nitrogen spatial pattern on carbon use and functional diversity in soil microbial communities

Nianxun Xi*, Juliette M.G. Bloor

INRA, UR874 Grassland Ecosystem Research Unit, 5 Chemin de Beaulieu, F-63039 Clermont-Ferrand, France

ABSTRACT

Impacts of climate change drivers on soil biota and biogeochemical cycling are of growing concern. Soil nitrogen (N) availability has the potential to mediate microbial responses to changes in precipitation via changes in microbial community composition, but the interactions between N spatial pattern and rainfall regime on soil microbial community structure and function remain unclear. We conducted an outdoor mesocosm experiment with a model grassland community to assess interactive effects of rainfall amount (control, −50% rainfall, +50% rainfall) and N spatial pattern (homogeneous, heterogeneous) on bacterial carbon (C) use and functional diversity using a community-level physiological profiling approach. Patchy N addition increased total bacterial C use and diversity of substrate use irrespective of rainfall treatment. Observed increases in microbial functional diversity contrasted with patch-induced decreases in plant community diversity in the study system. Neither bacterial C use nor diversity of C sources showed a significant response to rainfall regime or a significant rainfall × N pattern interaction. However, Principal Components Analysis showed clustering of bacterial substrate use across treatments, in line with different strategies of C use linked to both N spatial pattern and rainfall regime. Our findings suggest that bacterial substrate profiles are more sensitive to dry conditions when soil N is patchily distributed, with significant implications for soil organic C composition and storage potential in heterogeneous grasslands under future climatic conditions.

1. Introduction

Soil microorganisms play a key role for carbon (C) and nitrogen (N) cycling in terrestrial ecosystems via soil processes such as litter decomposition and N mineralization (Griffiths et al., 2003). However, growing evidence suggests that climate changes such as altered precipitation may modify microbial physiological activity, with cascading effects on biogeochemical cycling and climate-ecosystem feedbacks (Griffiths et al., 2003; Zhang et al., 2013). Improved understanding of soil microbial responses to environmental variation is needed for accurate prediction of ecosystem function in future climatic conditions (Treseder, 2008).

Rainfall regimes can have a strong influence on soil microbial ecophysiology, community structure and function due to direct effects on soil water availability (Williams and Rice, 2007; Schimel et al., 2007). Dry conditions are generally considered to reduce microbial activities and substrate-use, with stronger negative effects on bacteria compared to fungi (Manzoni et al., 2012). However, impacts of rainfall regimes and soil water on

microorganisms may be modulated by patterns of nutrient availability in soil. Recent work on grasslands shows that elevated N inputs consistently promote fast-growing bacterial taxa at the expense of fungi (Leff et al., 2015), and suggests that systems with low N inputs are more resistant to drought (de Vries et al., 2012). Localized inputs of N can also have both direct and indirect effects on microbial activity and community structure via changes in plant community composition and/or the amounts and types of plant C inputs to soils (Nunan et al., 2006; Orwin et al., 2009; Kuzyakov and Blagodatskaya, 2015). In theory, patchy N inputs should modify the sensitivity of microbial community structure and function to drought. In practice, interactive effects of rainfall regime and N spatial pattern on soil microbial community structure and function remain unexplored.

Here we investigate interactive effects of rainfall regimes and N spatial pattern on bacterial C use and functional diversity in a grassland mesocosm experiment, using a community-level physiological profiling approach. The estimation of bacterial functional diversity by C substrate use profiles has been reported to be a sensitive approach to detect changes in soil conditions (Pessi et al., 2012; Zhang et al., 2013), and is a useful tool for comparing microbial communities (Preston-Mafham et al., 2002). Previous

* Corresponding author.
E-mail address: nianxunxi@yahoo.com (N. Xi).

work in this study system has shown that patchy organic N inputs promote plant community dominance via shifts in plant species abundances irrespective of rainfall regimes (Xi et al., 2015). In the present study we examine whether bacterial responses mirror aboveground plant response patterns. We address three main hypotheses: (i) patchy N inputs modify bacterial community dominance based on C use; (ii) low rainfall decreases bacterial C use and functional diversity; (iii) patchy N inputs modify impacts of rainfall regimes on bacterial C use.

2. Material and methods

In September 2012, 36 experimental mesocosms were established outdoors at INRA-Clermont Ferrand, France (45°47′N, 03°05′E, 350 m a.s.l.). Each mesocosm comprised of a free-draining, steel box (50 cm diameter, 40 cm deep) filled with soil extracted from a nearby grassland (49.1% sand, 31.6% silt, 17.1% clay, 2.2% organic matter, C:N = 10.5, pH_{H2O} = 5.9). Mesocosms were planted with tillers/ramets of mature plants to create a grass-dominated plant community with ten species (see Xi et al., 2015 for full details).

A detailed description of experimental treatments is provided in Xi et al. (2015). In brief, three rainfall treatments based on precipitation during the growing season (control, Con; wet, +50% compared to control; dry, −50% compared to control) were crossed with two N pattern treatments (homogeneous, HOM; heterogeneous, HET), established by applying 50 kg N ha^{-1} in the form of Bovine serum albumen solution, a complex organic N form with limited lateral diffusion in soil. Application of N pattern treatments was carried out using protocols developed in previous field trials examining grassland responses to N heterogeneity (Xi et al., 2014). In the HOM treatments, 800 ml of dilute N solution was applied uniformly across the whole mesocosm. In the HET treatments, 50 ml of concentrated N solution was applied to a central 12.5 × 12.5 cm zone of each mesocosm, and 750 ml of distilled water was added to the remaining area. Total water and N addition were the same in all mesocosms during N application; the water in the N solution simulated a small rainfall event (3.2 mm). Rainfall manipulation occurred from the start of the plant growing season (30th March 2013) until final harvest (mid-June 2013), whereas N application took place on 16th April 2013 in line with local grassland management practices. Soil moisture content was lower in the DRY treatment and higher in the WET treatment compared to the control when N solution was added (Xi et al., 2015). Each of the six treatment combinations was replicated six times. Spatially-explicit plant measurements during the study period confirmed that the distinction between soil N availability in HET and HOM treatments was not compromised by root foraging or lateral diffusion of N solution (Xi et al., 2015).

In mid-June, at the peak biomass stage of vegetation, two soil cores (1.8 cm × 10 cm deep) were taken in the centre and edge of each mesocosm; these soil cores were pooled to provide a representative sample of the bacterial populations present at the mesocosm level. Two samples per treatment were combined to obtain three replicates, composite samples per treatment combination. All soil samples were sieved at 2 mm to remove plant roots and maintained at <5 °C prior to analysis. Community-level physiological profiles were characterized using Biolog Ecoplates (Biolog Inc., Hayward, CA, USA) following Zak et al. (1994). EcoPlates were incubated at 25 °C in the dark for 8 days and colour development of each well was determined daily by a series of optical density reading (OD at 590 nm) during incubation using a BioTek plate reader (BioTek Inc., USA) associated with Gen5 data analysis software. Carbon use for each substrate was measured as the area under the colour development profiles during the

Table 1

Treatment effects of rainfall and N spatial pattern on AWCD and Simpson's index based on C use. *F* values derived from analysis of variance are shown with degrees of freedom (*df*); significant effects (*P* < 0.05) are given in bold type.

Effect	df	AWCD	Simpson's index
Rainfall	2, 6	1.83	0.88
N pattern	1, 6	**56.78**	**24.37**
Rainfall × N pattern	2, 6	1.01	0.31

incubation period using the integration method (Hackett and Griffiths, 1997).

Average well colour development (AWCD) was determined as the mean carbon use for all 31 substrates. Simpson's dominance index (D) was calculated based on bacterial community C use following Magurran (2004), and provides a measure of functional diversity based on equitability of substrate use (higher values of D reflect the predominant use of a smaller number of substrates, whereas lower values of D reflect greater evenness of substrate use). Biolog data were analysed using a spit-plot two-way ANOVA (Quinn and Keough, 2002), with blocks as a random factor, rainfall treatments as a fixed whole-plot factor, and N treatments as a fixed sub-plot factor. Differences between treatments were determined with Tukey's HSD post-hoc tests. All data met assumptions of variance homogeneity and residual normality for performing ANOVA. Principal components analysis (PCA) was performed to examine patterns of microbial use of six broad classes of C substrate across treatments, based on the substrate classification by Preston-Mafham et al. (2002): amines, amino acids, carbohydrates, carboxylic acids, polymers and 'miscellaneous'. All analyses were performed using R and PCA analysis was conducted using the FactoMineR package (R Core Team, 2013).

3. Results

Bacterial C use and average well colour development increased over time, reaching a plateau by the 8th day of incubation in all treatments (data not shown). Integration across the incubation period indicated that AWCD was significantly higher in HET compared to HOM treatments (Table 1, Fig. 1a). In contrast, dominance of C use was significantly lower in HET compared to HOM treatments (Table 1, Fig. 1b). Neither AWCD nor Simpson's index showed significant responses to rainfall treatments or any rainfall × N spatial pattern interactions (Table 1).

The first two axes of the PCA analysis accounted for 88.8% of the total variance, and provided some evidence for treatment differentiation based on bacterial patterns of C utilization (Fig. 2). Homogeneous treatments were characterised by low carbohydrate use compared to HET treatments. Within HET treatments, average and above-average rainfall treatments were associated with high amine use, whereas dry conditions were associated with low amine use (Fig. 2).

4. Discussion

Water and N are key resources in grassland ecosystems, driving plant growth, microbial activity and biogeochemical cycling (Bloor and Bardgett, 2012). In the present study, we found that N spatial pattern modified bacterial C use and community dominance, with greater C use and diversity of C use (lower Simpson's index) in HET treatments compared to HOM treatments. These changes in substrate use indicate rapid responses of fast-growing, culturable bacteria to patchy N inputs, likely driven by patch-induced increases in plant production and plant-derived root exudates which stimulated bacterial growth and/or the proportion of active cells (Nunan et al., 2006). The observed increase in microbial