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Short communication

Soil microbial communities vary as much over time as with chronic warming and nitrogen additions

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ABSTRACT

We examined the effects of simultaneous warming and N additions on soil microbial biomass and community composition and assessed how the microbial community varied over seasonal and interannual timescales. The research took place at the Soil Warming and Nitrogen Addition (SWaN) Study at the Harvard Forest Long Term Ecological Research site. Nitrogen additions suppressed total biomass while warming increased biomass and shifted microbial community fingerprint. However, these responses were inconsistent over time, and seasonal and interannual differences in the microbial community were equal to or greater than treatment effects. For example, microbial biomass in ambient soils was 400% higher in 2008 than in 2010. This was comparable to the 524% difference in biomass between ambient and N addition plots in October, 2008. Our findings suggest that microbial communities can resist increased temperatures and N inputs for years to decades and that long term experiments are necessary to detect significant shifts in biomass and community composition.

Global change drivers such as climate warming and nitrogen (N) deposition are expected to alter soil microbial biomass and community composition with consequences for soil C and N cycles. Most studies examining the effects of increased soil temperature and N inputs on microbial biomass and community composition have occurred separately (e.g., DeForest et al., 2004; Schindlbacher et al., 2011). Many of these investigations have also focused on a single point in time (e.g., Frey et al., 2008) or a single growing season (e.g., Compton et al., 2004; Bradford et al., 2008). Yet soil microbial communities vary seasonally with fluctuations in temperature, moisture, and resource availability (Lipson et al., 1999; Bohlen et al., 2001; Waldrop and Firestone, 2006). This variability may result in differential seasonal responses to global change treatments. For example, soil warming might affect microbial biomass during summer drought differently than under winter snowpack. Over several years, chronically higher temperatures and N inputs may also impact the microbial community differently than just one year of warming and N additions (e.g., Rinnan et al., 2007).

The objectives of this study were to examine the effects of simultaneous warming and N additions on soil microbial biomass and community composition and to evaluate how the microbial community responded to experimental manipulations over time. The research took place at the Soil Warming × Nitrogen Addition (SWaN) study at the Harvard Forest Long Term Ecological Research (LTER) site in Petersham, Massachusetts, USA (42°50′ N, 72°18′W). The study area is in an even-aged, mixed hardwood stand with soils of the Gloucester series (Sandy-skeletal, mixed, mesic Typic Dystrudepts). Mean annual air temperature is 7 °C (ranges from −25 to 30 °C), and total annual precipitation is ~1100 mm (Boose et al., 2002). There are twenty-four, 3 × 3 m plots randomly assigned as ambient, N addition (N), warming (W), and warming × N (W × N). A subset of 12 plots was used in this study. Soils in W and W × N plots are continuously warmed 5 °C above ambient using buried heating cables at 10 cm depth. Nitrogen fertilization in the N and W × N plots occurs as equal doses of NH_4NO_3 applied throughout the growing season at a rate of 5 g N m^{-2} y^{-1} (Contosta et al., 2011).

To examine interannual variation in the microbial community, soils were sampled in autumn of 2006, 2008, and 2010. These collections followed peak leaf senescence and litter inputs to the forest floor. To determine seasonal variation, soils were sampled during January, April, July, and October, 2008, with dates chosen to

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coincide with conditions typical of winter, spring, summer, and autumn (Contosta et al., 2011). For each sampling, the litter layer (Oi) was brushed aside, and two 8 cm wide by 10 cm deep cores were removed from each plot, separated into the organic horizon (combined Oe/a; hereafter referred to as the O horizon, organic horizon, or organic soil) and mineral soils, and bulked by soil horizon. Bulked samples were passed through a 2 mm sieve to remove rocks and roots, and kept at 4 °C. Climatic and soil variables measured to examine environmental drivers of the microbial community are described in Supplemental Documents.

Microbial biomass and community composition was measured in both the O horizon and mineral soils using phospholipid fatty acid analysis (PLFA). Although it provides a general fingerprint of the microbial community, PLFA was chosen over higher resolution DNA and RNA methods due to several comparative advantages: 1) it is relatively rapid and inexpensive; 2) it has high precision between replicate samples; and 3) it has more statistical power than many nucleic acid techniques (Drenovsky et al., 2004, 2010; Ramsey et al., 2006; Frostegård et al., 2011). Most importantly, PLFA may have the lowest Type II error rate of common methods—including nucleic acid approaches—used to detect changes in microbial community composition with experimental treatments (Ramsey et al., 2006). It also may be more precise than molecular approaches such as quantitative PCR at measuring soil microbial biomass (Baldrian et al., 2013).

The microbial community in the O horizon was characterized with PLFA for all sample collection dates except October, 2006, when PLFA was only performed on the mineral horizon. Sieved, root-free soil was freeze-dried within 24 h of sampling and was stored at −80 °C. Microbial lipids were extracted from one gram of freeze-dried soil using a modified Bligh and Dyer (1959) procedure (White et al., 1979; Guckert et al., 1985) that isolated polar lipids and converted them to fatty acid methyl esters (FAMEs). Concentrations of FAMEs (nmol g^{-1} soil) were calculated from chromatographic peaks obtained from a Varian 3800 gas chromatograph (Varian, Inc, Santa Clara, California, USA) using an internal $C_{19:0}$ standard. Confirmation of FAME peak identities was obtained by GC-mass spectrometry (GC–MS) by Microbial Insights (Microbial Insights, Inc., Rockford, Tennessee, USA). The polyenoic unsaturated fatty acids 18:2ω6 and 18:1ω9c were fungal biomarkers (Bardgett et al., 1996; Bååth, 2003). Bacterial markers were Gram positive fatty acids (i15:0, a15:0, i16:0, i17:0, a17:0, 10Me16:0), Gram negative fatty acids (cy17:0, 18:1ω7c, cy19:0; Ekelund et al., 2003), Actinobacteria (10Me16:0; Frostegård et al., 1993) and general bacterial markers (15:0, 16:1ω7c, and 16:1ω7t; Leckie et al., 2004). Total, fungal, bacterial, Gram positive, and Gram negative lipid biomass were determined by adding all markers within each functional group. The fungal:bacterial (F:B) ratio was the lipid biomass of fungi divided by bacteria. Additional ratios were calculated to assess whether warming altered membrane fluidity, thereby obscuring shifts in microbial biomass and community composition (Wixon and Balser, 2013). These included the lipid ratio of similar monounsaturated fatty acids with different branching patterns (i.e., 16:1ω7t/16:1ω7c) and the ratio of cyclopropyl fatty acids to their precursors [i.e., (cy17:0 + cy19:0)/(16:1ω7c + 18:1ω7c); Kaur et al., 2005; Wixon and Balser 2013]. Neither of these ratios changed with warming and/or N addition treatments over interannual or seasonal timescales. As a result, any changes reported in the microbial community are considered actual shifts in lipid biomass and not changes in membrane fluidity due to physiological stressors such as elevated temperature.

All statistical analyses were conducted in R 3.0.1 (R Core Development Team, 2009). Relationships between the microbial community and environmental drivers were determined with correlations (for biomass) and fitting environmental vectors onto spatial ordinations (for community composition; see below). These methods are outlined in more detail in Supplemental Documents, and results are presented in SD Table 1. Mixed effects models (*nlme* package; Pinheiro et al., 2009) assessed changes in biomass given experimental treatments and time. Response variables were total, fungal, bacterial, actinobacterial, Gram positive, and Gram negative lipid biomass, and the F:B ratio. Each variable was log-transformed to meet normality and homoscedasticity assumptions, but data in figures are presented in their original scale. Differences between soil horizons obscured variation over time and in response to experimental treatments. As a result, separate analyses were conducted for O horizon and mineral soils both within 2008 (seasonal models) and among years 2006–2010 (interannual models). Main effects were warming, N additions, year (for interannual models), and month (for seasonal models), and all possible interactions. Inclusion of random effects, autocorrelation, and variance structures, and significance of main effects and interactions were determined using the protocol in Zuur et al. (2009), with addition details provided in Supplemental Documents and SD Tables 2 and 3.

Changes in microbial community composition were evaluated with spatial ordinations and permutation tests in the *vegan* package (Oksanen et al., 2010). These analyses were conducted separately for organic and mineral horizons and for interannual and seasonal timeframes. Data were relative abundances of each lipid biomarker that were converted into a dissimilarity matrix with the Bray distance. For spatial ordinations, nonmetric multidimensional scaling (NMDS) was performed using *metaMDS*. Permanova (Anderson, 2001) was then applied to determine the effects of warming, N additions, year (interannual models), and month (seasonal models) on microbial community composition using *adonis*. Permanovas included the same response variables, main effects, and interactions as for mixed effects models, and *F*-statistics and *p*-values were obtained from 999 permutations.

The effects of warming and/or N additions on the microbial community tended to be ephemeral and differed by soil horizon. Fig. 1 shows box plots of the variation in total lipid biomass in organic and mineral soils over interannual and seasonal timescales, where a lack of overlap between sample medians typically suggests a statistically significant difference between or among treatment groups (Krzywinski and Altman, 2014). Changes in fungal, bacterial, actinobacterial, Gram positive, and Gram negative, and lipid biomass with experimental treatments and time were similar to those of total lipid biomass and are shown in Table 1 and SD Figs. 1–5).

In the O horizon, the most significant treatment effect over interannual and seasonal timescales occurred in October, 2008, when N additions reduced total microbial lipid biomass 524% relative to ambient plots (Fig. 1, Table 1). Warming alone did not alter biomass, but the combination of warming × N somewhat mitigated the negative effect of N in October, 2008, reducing total biomass by only 186%. Warming also reduced the F:B ratio independently of sampling month (by 16%), but only marginally decreased the F:B seasonally across all sampling years (by 17%, Table 1, SD Fig. 6).

In the mineral soil, there were no treatment differences over an interannual timeframe. However, there was a 64% increase in total lipid biomass in warmed plots relative to ambient plots only in July, 2008 (Fig. 1, Table 1). Within 2008, warming and N additions also reduced the F:B ratio by 10% for each treatment (SD Fig. 7, Table 1). In addition, warming altered community composition in mineral soils, but this shift was seasonal and was most pronounced in April and July, 2008 (Fig. 2, Table 1).

Although reduced biomass and lower F:B ratios fit with the findings of other studies, these results were idiosyncratic, occurring

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