Multiple Climate Change Factors Interact to Alter Soil Microbial Community Structure in an Old-Field Ecosystem

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Climate change has the potential to alter both the composition and function of a soil’s microbial community, and interactions among climate change factors may alter soil communities in ways that are not possible to predict from experiments based on a single factor. This study evaluated the direct and interactive effects of three climate change factors—elevated CO₂, altered amounts of precipitation, and elevated air temperature—on soil microbial communities from an old-field climate change experiment being conducted at Oak Ridge, TN. Soil microbial community composition and biomass were determined by phospholipid fatty acid (PLFA) and neutral lipid fatty acid composition. We found that the interactive effects of precipitation and temperature treatments, as well as the interactive effects of precipitation and CO₂ treatments, had significant impacts on microbial community composition. We found that total soil PLFA concentration, a measure of microbial biomass, was greater in the low-precipitation treatments, especially when low precipitation was combined with ambient CO₂ concentrations or ambient temperature. Ordination analysis indicated that temperature was the most significant predictor of shifts in the soil microbial community composition, explaining approximately 12% of the variance in relative abundance of PLFA biomarkers. The elevated-temperature treatment increased the abundance of Firmicutes (low-guanine–cytosine Gram positive) and decreased the abundance of Gram-negative bacteria. Elevated temperature also reduced the abundance of the arbuscular mycorrhizal fungi PLFA biomarker 16:1ω5 and saprophytic fungal PLFA biomarker 18:2ω6,6. Overall, our data indicate that the interactions among climate change factors alter the composition of soil microbial communities in old-field ecosystems, suggesting potential for changes in microbial community function under predicted future climate conditions.

Abbreviations: AMF, arbuscular mycorrhizal fungi; BrSFA, branched, saturated fatty acid; BSI, bacterial stress index; CyFA, cyclopropyl fatty acid; GC, guanine and cytosine; MUFA, monounsaturated fatty acid; NLFA, neutral lipid fatty acid; OCCAM, Old-Field Community, Climate, and Atmosphere Manipulation; PLFA, phospholipid fatty acid.

Microbial communities are an integral component of biogeochemical processes influencing the cycling of both C and nutrients in soils (Buckley and Schmidt, 2003; Pendall et al., 2004; Bardgett et al., 2008). The composition and abundance of the soil microbial community influences the kinds of organic and inorganic materials that can be utilized as substrates, as well as the reaction rate of substrate utilization (e.g., Waldrop et al., 2000). Furthermore, the potential exists for changes in both the composition and function of various components of the soil microbial community in response to changes in climate, such as rising temperatures, increasing atmospheric CO₂, and altered precipitation patterns (e.g., Pendall et al., 2004; Compant et al., 2010). While the direct effects of individual climatic change factors on soil communities have been demonstrated in a number of studies (e.g., Monteagle et al., 2002; Kandeler et al., 2008; Austin et al., 2009), we know less about how these climate drivers may interact to shape soil communities (Chung et al., 2007; Castro et al., 2010; Antoninka et al., 2009; Kardol et al., 2010b, 2011).
Elevated temperature can directly alter the soil microbial community function by affecting temperature-sensitive microbial enzyme activity (von Lützow and Kogel-Knabner, 2009). Elevated temperature can also influence soil microbial abundance and composition by altering net primary production and therefore the pool of available substrates utilized for microbial growth. Elevated temperature also increases the evaporative flux of water from the soil and therefore indirectly affects microorganisms through soil drying (Pendall et al., 2004; Zogg et al., 1997; Filella et al., 2004). The effects of elevated temperature on the total microbial biomass are variable (Zogg et al., 1997; Pendall et al., 2004), and groups of microorganisms differ in their responses to elevated temperature. For example, Firmicutes (low guanine and cytosine [GC], Gram positive) and Actinobacteria (high GC, Gram positive) have been observed to respond positively to elevated temperature, while Gram-negative bacteria respond negatively (Bardgett et al., 1999; Zogg et al., 1997). The response of the mycorrhizal fungal community to elevated temperature is variable and will probably be closely linked to the productivity and C allocation responses of host plants (Bentivenga and Hetrick, 1992; Rillig et al., 2002; Heinemeyer and Fitter, 2004).

Rising atmospheric CO₂ concentrations can alter the biomass and composition of soil microbial communities indirectly via increases in plant biomass production, increased C inputs from litterfall and root turnover, and mitigation of soil drying via reduced stomatal conductance of plants (Hu et al., 1999; Norby et al., 2001). Given the positive relationship between plant productivity and microbial biomass across many different soil types (Schimel, 1986; Burke, 1989), increases in plant productivity under elevated CO₂ are expected to result in increased microbial biomass (e.g., Zak et al., 2000). Increased plant productivity, litter inputs, and rhizodeposition, as well as changes in plant community composition, can change the biochemical characteristics and quantity of resources that plants deliver to the soil under elevated CO₂ (e.g., Kandeler et al., 2008), differentially affecting groups of microorganisms. For example, increased rhizodeposition of plants grown under elevated CO₂ may increase the availability of low-molecular-weight C substrates, favoring Gram-negative bacteria over Firmicutes or Actinobacteria (Kandeler et al., 2008; Milchunas et al., 2005; Drissner et al., 2007).

Changes in precipitation can affect the composition and functioning of the soil microbial community through differential drought tolerance among taxonomic and functional groups of microorganisms. For example, more drought-tolerant microorganisms such as fungi, Firmicutes, and Actinobacteria usually benefit from dry conditions (Griffin, 1969; Drenovsky et al., 2004; Sylvia et al., 1998), while the abundance of Gram-negative bacteria usually declines under soil drying (Harris, 1981; Nesci et al., 2004; Schimel et al., 2007). These changes lead to an increased ratio of fungi to bacteria under dry conditions, as measured by an increased microbial C/N ratio (Jensen et al., 2003) and increased fungal/bacterial PLFA ratio (Drenovsky et al., 2004), resulting in a shift in community composition, which has important implications for nutrient cycling and decomposition processes.

While much insight has been gained with regard to how changes in climate affect soil microbial communities, we have much to learn about how interactions among these variables might affect microbial communities. Interactive climate change effects on soil moisture (Dermody et al., 2007), soil temperature, and plant-mediated shifts in resource inputs such as changes in quantity and biochemical characteristics of litter or allocation of C to roots (Dukes and Hungate 2002) suggest a potential for downstream effects on the composition of soil communities (Hungate et al., 1996; Kardol et al., 2010b, 2011). We took advantage of a multifactor climate change experiment that manipulated the atmospheric CO₂, air temperature, and precipitation in an old-field community to examine how multiple climate change factors directly and interactively shape soil communities. We predicted that the availability of soil moisture would have the largest influence on soil microbial communities and hence the precipitation treatments would moderate the impacts of warming and elevated atmospheric CO₂ on the soil community composition.

**MATERIALS AND METHODS**

**Site Description**

The Old-Field Community, Climate, and Atmosphere Manipulation (OCCAM) experiment was established in 2002 at the Oak Ridge National Laboratory National Ecological Research Park in Oak Ridge, TN (35°54′12″ N, 84°20′22″ W). Soils at this site are developed in well-drained, slightly acidic, floodplain alluvium and are classified as Captina silt loam (a fine-silty, siliceous, active, mesic Typic Fragiaclude) (Edwards and Norby, 1999). The average soil pH at the OCCAM site is 5.8 across treatments. Before establishment of the experimental plots, the existing vegetation was killed using glyphosate herbicide [N-(phosphonomethyl)glycine], and dead plants as well as aboveground meristems were removed by scraping away the top 1 cm of soil using a hoe (Engel et al., 2009). In August 2002, following removal of the existing vegetation, seedlings of seven plant species common to old-field communities in the southeastern United States were transplanted into plots. These species included Plantago lanceolata L., a herbaceous forb; Andropogon virginicus L., a cespitose C₄ bunchgrass; Festuca pratensis Huds. subsp. apennina (De Not.) Hegi, syn. F. labor L. var. apennina (De Not.) Hack., a C₃ bunchgrass; Dactylis glomerata L., a C₃ bunchgrass; Trifolium pratense L., a herbaceous legume; Solidago canadensis, a herbaceous forb; and Lespedeza cuneata (Dum. Cours.) G. Don, a N₂-fixing dwarf shrub (see Engel et al., 2009; Kardol et al., 2010a,b).

This experimental facility has been extensively described elsewhere (Garten et al., 2008; Engel et al., 2009), but treatments are described briefly below. Open-top chambers (4-m diameter) with rain-exclusion shelters were constructed on 12 plots in each of three blocks. Treatments of ambient and elevated (3°C above ambient) air temperature, and differential irrigation (dry and wet plots) began in May 2003. The elevated CO₂ treatment was achieved by injecting pure CO₂ into the chamber plenum (Engel et al., 2009). The daytime CO₂ concentration in the ambient CO₂ treatment averaged 395 µmol mol⁻¹ in 2004 and 394 µmol mol⁻¹ in 2005, and daytime CO₂ concentration in elevated CO₂ treatment averaged 703 µmol mol⁻¹ in 2004 and 707 µmol mol⁻¹ in 2005 (warming.
Ornol.gov/OCCAM/data.html; verified 15 Aug. 2011). Temperature treatments were achieved through the use of evaporative coolers and in-line heating coils (Norby et al., 1997). The annual average air temperature in the ambient temperature treatment was 14.89 ± 0.17°C in 2004 and 14.96 ± 0.11°C in 2005, and the annual average air temperature in the elevated temperature treatments was 17.56 ± 0.18°C in 2004 and 17.19 ± 0.53°C in 2005 (warming.ornol.gov/OCCAM/data.html; verified 15 Aug. 2011). The CO2 and temperature treatments were imposed at a whole-plot level, and each whole plot was divided into two 6.3-m² split plots that received either wet or dry irrigation treatments. Rainwater was collected onsite and added weekly to maintain the dry (2 mm wk⁻¹) and wet (25 mm wk⁻¹) split plots. The average annual precipitation at the field site is 136 cm, approximately 26 mm wk⁻¹ (Garten et al., 2009). Therefore, the wet treatment represents standard precipitation conditions at Oak Ridge, and the dry treatment represents a significant decrease in precipitation, which reduced the soil volumetric water content in the top 15 cm from 26.2% in the wet treatments to 21.7% in the dry treatments (Wan et al., 2007). The absolute humidity in the plots was not affected by treatment (Wan et al., 2007). Treatments are designated as: ECAT, ACAT, ECET, and ACET, where EC is elevated CO2, AC is ambient CO2, ET is elevated temperature, and AT is ambient temperature.

Soil Sampling and Analysis

Soil samples were collected in August 2004, October 2004, and June 2005 (0–15 cm) using a 5-cm-diameter soil corer. Three samples were collected at random locations within each split plot and stored at −20°C. Before processing, the samples were thawed, sieved to 4 mm, and roots were removed. The soils were freeze-dried (−50°C, 8 GPa) for 48 h in a Labconco Freezezone 4.5 freeze-drier (Labconco, Kansas City, MO), subsamples were composited to yield one sample per subplot per sampling date, and the composited samples were stored at room temperature until laboratory analysis of PLFA and neutral lipid fatty acid (NLFA) contents.

Lipids were extracted from a 2-g subsample of the composited, freeze-dried soil in a single-phase mixture of chloroform, methanol, and phosphate buffer (pH 7.4) at a ratio of 1:2:0.8, using an adaptation of the method described by Bligh and Dyer (1959). After 3 h, water and chloroform were added to separate the mixture into polar and nonpolar phases. Total lipids were extracted from the chloroform phase and phospholipids were separated from the neutral lipids using silicic acid column chromatography (Allison et al., 2005). Phospholipids and neutral lipids were then saponified and the resulting fatty acids were methylated using a mild alkaline solution to form fatty acid methyl esters (FAMEs), and samples were frozen until analysis via gas chromatography.

Before gas chromatography analysis, samples were thawed and dissolved in a 24 mg L⁻¹ solution of FAME 19:0 dissolved in hexane (Matreya, Pleasant Gap, PA). This known concentration of FAME 19:0 was used as an internal standard to quantify the concentration of PLFAs or NLFAs detected in the biological samples. Fatty acids were separated by capillary gas chromatography, using an HP 6890 gas chromatograph equipped with an HP7683 autosampler (Agilent Technologies, Palo Alto, CA). A 30-m HP-5MS column was used, with H2 as the carrier gas at a constant flow rate of 4 mL min⁻¹. For each sample, a 1-µL splitless injection was made, with the inlet temperature set at 230°C and the inlet purged at 47 mL min⁻¹ 0.75 min after injection. The oven temperature was held at 80°C for 1 min, increased at a rate of 20°C min⁻¹ up to 155°C, and then increased at a rate of 5°C min⁻¹ to a final temperature of 270°C. The oven temperature was then held at 270°C for 5 min. Individual fatty acids were detected by flame ionization at 350°C. Fatty acids were identified by retention time in comparison to known standards and were quantified using a known concentration of FAME 19:0 as an internal standard.

Fatty acid nomenclature is in the form of A:B:C, where A is the number of C atoms in the chain, B is the number of double bonds, and C is the position of the double bond from the methyl end of the PLFA (Tunlid and White, 1992; Allison and Miller, 2005). A cis geometry is indicated by the suffix c. The prefixes i, a, and me refer to iso, anteiso, and midchain methyl branching, respectively, and cy indicates a cyclopropyl ring structure.

Data Analysis

Individual PLFAs were summed to give an estimate of the total microbial biomass (µmol kg⁻¹ soil) in each sample. The average concentration of each individual PLFA is shown for all treatment combinations and sampling dates in Supplementary Table 1. To make inferences on the effects of climate change treatments on functionally similar microbial groups, we grouped signature fatty acids into biomarkers for the following microbial groups: Firmicutes, Gram-negative bacteria, Actinobacteria, arbuscular mycorrhizal fungi (AMF) and saprophytic fungi (Table 1). We further evaluated the energy storage of the AMF community using NLFAs (Olsson et al., 1997). Specifically, we estimated the total NLFA concentration for AMF species of the genera Glomus sensu lato using NLFA 16:1π5c (Graham et al., 1995; Olsson et al., 1999). We estimated the total NLFA concentration for AMF species of the genus Gigaspora as NLFA 22:1π9 + NLFA 20:1π9 (Graham et al., 1995; Bentivenga and Morton, 1996; Madan et al., 2002). We estimated treatment effects on the Gigaspora/ Glomus species ratio as the ratio of these two sums of NLFAs. We estimated the ratio of Firmicutes to Gram-negative bacteria in each sample as the ratio of branched, saturated fatty acids to monounsaturated fatty acids (BrSFA/MUFA; Vestal and White, 1989; White et al., 1993). The degree of physiological stress experienced by the Gram-negative bacterial community was estimated using the bacterial stress index (BSI) for each sample: BSI = 17·0 + 19·0/16·17c + 18·167c (Guckert et al., 1986; Bossio et al., 1998). A further estimate of Gram-negative physiological stress was made using the ratio of cyclopropyl fatty

| Table 1. Phospholipid fatty acids (PLFAs) signature assignments used in this study. |
|---------------------------------------|----------------|----------------|
| Group                                | Biomarker PLFAs |
| Actinobacteria (high-GC gram positive)| 10me16:0†, 10me18:0† |
| Bacteria                             | 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 22:0 |
| Gram-negative bacteria               | 16:1, 17:0, 18:1 |
| Firmicutes (low-GC Gram positive)    | 15:0, 16:0, 17:0 |
| Fungi Saprophytic                    | 18:2, 9c, 18:1, 19c |
| Arbuscular mycorrhizal fungi         | 16:1, 5c, 20:1, 9c |
| † Zelles (1999).                     |                |
| ‡ Zak et al. (1996).                 |                |
| § Frostegård and Båth (1996).        |                |
| ¶ Frostegård et al. (1993).          |                |
| ‡‡ Olsson (1999).                    |                |
| †† Madan et al. (2002).              |                |
Fig. 1. Total phospholipid fatty acid (PLFA) concentrations (least squares means ± standard error of the means averaged across August 2004, October 2004, and June 2005; AT = ambient temperature, ET = elevated temperature, AC = ambient CO2, EC = elevated CO2). Direct and interactive effects of treatments that were significant at the P ≤ 0.05 level are indicated, along with their P values.

We tested the across-sampling-date effects of the treatments using Monte Carlo permutation tests (999 permutations) restricted for split-plot design, i.e., records at different dates from the same plots (split plots) were not permuted, while whole plots were freely exchangeable (Lepš and Šmilauer, 2003). Samples were permuted within blocks. Individual treatment and interaction effects (marginal effects) were tested by manual selection of each individual term. Redundancy analyses were performed using CANOCO, version 4.5 (ter Braak and Šmilauer, 2002).

**RESULTS**

**Treatment Effects on Phospholipid Fatty Acid Concentrations in Microbial Groups**

The total soil PLFA concentration was higher in dry treatments than wet treatments under ambient temperature, but precipitation had no effect on the PLFA concentration under elevated temperature ($F_{1,40} = 7.27, P = 0.01$; Fig. 1; Supplementary Table 2). Additionally, there was a significant interaction between CO2 and precipitation treatments, where total PLFA concentrations were higher in dry treatments exposed to ambient CO2, but precipitation had no effect on total PLFA concentrations under elevated CO2 ($F_{1,40} = 4.07, P = 0.05$; Fig. 1). Interestingly, total PLFA concentrations and concentrations of PLFA accounted for by specific microbial groups did not differ significantly among the August 2004, October 2004, and June 2005 collection dates, and there were no significant interactive effects of sampling date and treatment on total PLFA or PLFA of specific microbial groups (Supplementary Table 2).

Similar to total PLFA (Fig. 1), the amount of PLFA accounted for by bacterial markers was higher in dry treatments than wet treatments exposed to ambient temperature, but precipitation had no effect on total bacterial PLFA concentrations under elevated temperature ($F_{1,40} = 7.98, P < 0.01$; Fig. 2A). Additionally, total bacterial PLFA concentrations were higher in dry treatments exposed to ambient CO2, but precipitation had no effect on total PLFA concentrations under elevated CO2 ($F_{1,40} = 4.2, P < 0.05$; Fig. 2A).

The temperature × precipitation interaction on total bacterial PLFA appeared to be driven by both Gram-negative bacteria and Firmicute PLFA concentrations. Firmicute PLFA concentrations were higher in the dry treatment than the wet treatment under ambient temperature, but precipitation had no effect on Firmicute PLFA concentrations under elevated temperature ($F_{1,40} = 7.39, P < 0.01$; Fig. 2B). Gram-negative bacterial PLFA concentrations showed similar interactive effects of temperature and precipitation ($F_{1,40} = 7.46, P < 0.01$; Fig. 2C). The BrSFA/MUFA ratio was significantly greater in the elevated temperature treatment than the ambient temperature treatment, indicating that the elevated temperature increased the abundance of Firmicutes relative to Gram-negative bacteria ($F_{1,6} = 10.94, P = 0.02$; Fig. 3). Additionally, the BrSFA/MUFA ratio was significantly greater in dry plots than wet plots, indicating that reduced precipitation increased the abundance of Firmicutes relative to Gram-negative bacteria ($F_{1,40} = 10.05, P = 0.003$; Fig. 3).

The BSI was significantly higher under elevated relative to ambient temperature treatments ($F_{1,6} = 12.0, P = 0.01$; Fig. 2D). The BSI was also significantly higher in dry treatments than wet treatments ($F_{1,40} = 8.46, P < 0.01$; Fig. 2D); however, a significant CO2 × precipitation interaction revealed that the effect of precipitation was only significant in combination with ambient CO2 ($CO_2 \times H_2O F_{1,40} = 5.18, P = 0.03$; Fig. 2D). Similar results were found for the CyFA/MUFA ratio, which was significantly higher in the elevated temperature treatment than the ambient temperature treatment ($F_{1,6} = 8.08, P = 0.03$; results not...
Additionally, there were significant interactive effects of CO₂ and precipitation on this ratio, where the CyFA/MUFA ratio was significantly greater in dry treatments than wet treatments under ambient CO₂, but there was no effect of precipitation on the CyFA/MUFA ratio under elevated CO₂ ($F_{1,40} = 6.52, P = 0.01$; results not shown).

Both temperature and CO₂ interacted with precipitation to alter Actinobacteria PLFA concentrations (Supplementary Fig. 2).
There was also a significant CO2 × precipitation interaction (F1,40 = 3.53, P = 0.07; Fig. 2H). There were no significant single or interactive effects of the climate change treatments or sampling date on the fungal/bacterial PLFA ratio (data not shown).

Multivariate Analysis

Together, the climate change treatments explained 21% of the total variation in PLFA composition. Temperature had the strongest effect on PLFA composition (explaining almost 12% of the variation, Table 2), as indicated by its correlation with the first redundancy analysis axis (Fig. 4). The temperature effect was dependent on the CO2 treatment (Table 2). The ordination diagram shows that PLFA biomarkers for *Firmicutes* (i15;0 and i15:0) and *Actinobacteria* (10me18:0 and 10me16:0) were associated with elevated temperature treatments, while PLFA biomarkers for AMF (16:1α5 and 20:1α9) and Gram-negative bacteria (16:1α7 and 18:1α7, which Graham et al. [1995] and others have also described as commonly found in AMF) were associated with ambient temperature treatments (Fig. 4). The precipitation treatment had fewer significant effects than the temperature treatment; however, there was a significant CO2 × precipitation effect explaining about 4% of the variation (Table 2), with wet, elevated CO2 treatments separating from dry, ambient CO2 treatments. Most notably, the i15:0 biomarker for *Firmicutes* was strongly associated with dry, ambient CO2 treatments (Fig. 4).

**DISCUSSION**

Significant interactive effects of temperature and precipitation as well as interactive effects of CO2 and precipitation impacted the biomass and composition of the soil microbial community in our experiment. Surprisingly, and contrary to what we predicted, total PLFA concentrations were higher in dry than wet treatments, particularly when combined with ambient temperature. These results were somewhat surprising, given that previous work in this system showed that, rather than responding to precipitation treatments, bacterial abundance (as indicated by total copies of rRNA genes) responded primarily to ambient temperature treatments. These results were somewhat surprising, given that previous work in this system showed that, rather than responding to precipitation treatments, bacterial abundance (as indicated by total copies of rRNA genes) responded primarily to ambient temperature treatments.

### Table 2. Results from redundancy analysis (RDA) testing the effect of treatments CO2, temperature (T), H2O, and their interactions on the relative abundance of phospholipid fatty acid biomarkers. Explained variance (marginal effects) is the sum of all canonical eigenvalues.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>F ratio</th>
<th>P value</th>
<th>Explained variance %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO2</td>
<td>1.92</td>
<td>0.16</td>
<td>2.5</td>
</tr>
<tr>
<td>T</td>
<td>9.99</td>
<td>&lt;0.01</td>
<td>11.8</td>
</tr>
<tr>
<td>H2O</td>
<td>2.76</td>
<td>&lt;0.01</td>
<td>3.6</td>
</tr>
<tr>
<td>CO2 × T</td>
<td>2.81</td>
<td>0.02</td>
<td>3.7</td>
</tr>
<tr>
<td>CO2 × H2O</td>
<td>2.85</td>
<td>0.01</td>
<td>3.7</td>
</tr>
<tr>
<td>H2O × T</td>
<td>1.64</td>
<td>0.21</td>
<td>2.2</td>
</tr>
<tr>
<td>CO2 × H2O × T</td>
<td>0.82</td>
<td>0.50</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Significant at P ≤ 0.05 based on permutation tests restricted for sampling date and block.
to the interactive effects of \( CO_2 \) and temperature, while fungal abundance (as indicated by total copies of rRNA genes) responded to the direct effects of temperature (Castro et al., 2010). Nematode data from this experiment, however, suggests that grazing of the soil microbial community by nematodes could explain some of the reduction in total microbial biomass under wet conditions (Kardol et al., 2010b). The total PLFA values averaged across our three sampling dates were negatively correlated with total nematode numbers in soils collected from OCCAM in October 2007; however, it is important to note that this value included plant-feeding nematodes as well as microbial-feeding nematodes (Kardol et al., 2010b; Pearson’s \( r = -0.70, P = 0.06 \)).

The interactive effects of temperature and precipitation on bacterial-feeding nematodes may explain why the concentrations of PLFA biomarkers for the total bacterial community were greater in the dry, ambient temperature treatments. The number of bacterial-feeding nematodes in this soil community decreased in dry treatments, but this effect was not apparent in elevated \( CO_2 \) and ambient temperature treatments (Kardol et al., 2010b). While we did observe an increase in PLFA concentration for bacterial markers in the dry treatment, this effect was magnified in the ambient temperature treatments. Similar to total PLFA, we found a strong negative correlation between total bacterial PLFA averaged across our three sampling dates and the number of bacterial-feeding nematodes in soils collected from this field site in October 2007 (Kardol et al., 2010b; Pearson’s \( r = -0.82, P = 0.01 \)). Firmicutes and Actinobacteria are known to be inherently resistant to drying due to their strong, interlinked peptidoglycan cell walls and their ability to synthesize osmotic protectants (Schimel et al., 2007; Sylvia et al., 1998), which is consistent with our observations that the dry treatment resulted in increased PLFA concentrations in both of these microbial groups. The strong negative correlation between soil bacterial PLFA concentrations and nematode abundance (Kardol et al., 2010b) indicates that top-down control in response to reduced grazing by nematodes, rather than a direct physiological response to climate change treatments, may have influenced the bacterial community response.

Generally, Gram-negative bacteria are poorly adapted to drought because they rely on inducible osmotic protectants to prevent desiccation (Schimel et al., 2007). During times of metabolic stress, Gram-negative bacteria convert cis-monoenoic fatty acids to CyFAs, which allows greater membrane stability (Guckert et al., 1986; Bossio et al., 1998). Consistent with previous findings (Steinberger et al., 1999), the BSI was higher in elevated relative to ambient temperature treatments. This was probably a response to the combined stresses of increasing temperature and the reduction in soil moisture that occurred under the elevated temperature treatment in our experiment (Dermody et al., 2007). Elevated temperature also increased the CyFA/MUFA ratio, indicating a starvation...
response or a shift to stationary phase growth (Guckert et al., 1986). This observation is consistent with treatment effects on the BSI.

We also measured several significant interactive effects of precipitation and CO₂ (Supplementary Table 2; Fig. 1–2 and 5). The total PLFA was greater in dry than wet treatments, but this effect was not significant under elevated CO₂ (Table 2; Fig. 1). Under ambient CO₂, the concentration of *Actinobacteria* PLFA was significantly higher in dry treatments but under elevated CO₂ wet and dry treatments did not differ. These patterns probably resulted from the mitigation of soil drying under elevated CO₂, as previously documented at our experimental site (Dermody et al., 2007). Mitigation of soil drying by elevated CO₂ may have also contributed to a lack of precipitation effects on bacterial-feeding nematodes (Kardol et al., 2010b) in the elevated CO₂ treatment at OCCAM, which, in turn, probably contributed to the interactive effects of CO₂ and precipitation on bacterial PLFA concentrations. This trend was also observed in total bacterial PLFA and *Firmicutes* PLFA, although the interactive effects of CO₂ and precipitation on *Firmicutes* were not significant.

Similar to the results for total PLFA, the BSI was higher in dry than wet treatments but only under ambient CO₂. This previously noted interaction between CO₂ and precipitation demonstrates a novel mechanism by which mitigation of soil drying by elevated CO₂ may decrease the degree of physiological stress experienced by Gram-negative bacteria due to reduced precipitation.

Multivariate analysis indicated that elevated temperature increased the relative abundance of *Firmicutes* and decreased the relative abundance of AMF, saprophytic fungi, and Gram-negative bacteria. These results are supported by the trend of the BrsFA/MUFA ratio to increase in the elevated temperature treatment, indicating an increased ratio of *Firmicutes* to Gram-negative bacteria. It was somewhat surprising that the wet treatment did not increase the AMF PLFA concentration, given that the wet treatment increased the plant community aboveground biomass at OCCAM by 80% (Kardol et al., 2010b). Our findings suggest, however, that climate change factors may have indirectly affected the fungal community via changes in nematode abundance. The saprophytic fungal PLFA concentration was negatively correlated with the density of fungal-feeding nematodes in the soil at this field site (Kardol et al., 2010b; Pearson's r = −0.63, P = 0.09).

In addition to altering the abundance of fungal-derived lipids in the soil, environmental change may also alter the allocation pattern of energy-rich lipids to storage structures and spores of AMF (Bäath, 2003; Gavito and Olsson, 2003). We demonstrated that the dry treatment caused an increase in the *Gigaspora/Glomus* NLFA ratio (Fig. 5). Reduced soil moisture in the dry plots probably preferentially stimulated the allocation of host photosynthesis to storage lipids and the production of spores in members of the AMF genus *Gigaspora*. Although variation exists for the kinds of neutral lipids that various AMF groups use as storage reserves, there is a tendency for members of the Glomaceae to accumulate the neutral lipid 16:10c5, whereas members of the genus *Gigaspora* accumulate 20:169 and 22:169 (Graham et al., 1995; Olsson, 1999; Madan et al., 2002). We found that the NLFA that accumulated under low precipitation were those NLFA associated with members of the various species that comprise the genera associated with *Glomus sensu stricto* and *Scutellospora*. Even so, the NLFA that accumulated in association with AMF species belonging to the genus *Gigaspora* showed a significantly larger increase in the allocation of energy to spore production in response to the reduced precipitation treatment (Fig. 5).

Our results indicate that the interactions of reduced precipitation, elevated temperature, and elevated CO₂ can significantly alter the biomass and composition of soil microbial communities. The strength of these interactive effects was demonstrated by the observation that the dry treatment caused a 48% increase in total PLFA when combined with ambient CO₂ and ambient temperature but did not affect the total PLFA in other treatment combinations (Fig. 1). This experiment provides unique evidence linking the previously reported reduction of soil drying under elevated CO₂ (Dermody et al., 2007) to the mitigation of dry treatment effects on soil microorganisms—for example, the desiccation-resistant *Actinobacteria* PLFA concentration increased in dry treatments but this effect was not significant under elevated CO₂ where soil drying was reduced. Additionally, we demonstrated that previously reported changes in soil nematode populations in our experiment (Kardol et al., 2010b) were negatively correlated with the PLFA concentrations of various microbial groups. These results suggest that, in addition to directly affecting microbial populations on the physiological level, elevated CO₂, elevated temperature, and altered precipitation may affect microbial community biomass through top-down control. Recently, De Deyn et al. (2011) showed that PFLA biomarkers for individual microbial groups differentially responded to changes in plant community structure. Therefore, it is probable that climate-change-induced shifts in the plant community also have contributed to the observed changes in microbial community composition in our experiment. Kardol et al. (2010b) found that the precipitation treatments explained the greatest amount of variation (12%) in the plant community composition at OCCAM—more than temperature or CO₂ treatments—and that the dry treatment shifted plant cover dominance from *Lespedeza cuneata* to *Festuca pratensis*. Interestingly, they also found that enzyme activities in soils under *L. cuneata* were reduced relative to soils under *F. pratensis*, suggesting lower microbial biomass beneath *L. cuneata* (Kardol et al., 2010b). These data suggest that the shift to *F. pratensis* dominance that occurred under the dry treatment at OCCAM may have played a role in the increased PLFA concentrations under the dry treatment that were observed in this study (Fig. 1). Changes in the relative abundance of individual microbial groups suggest that, with time, climate change factors may alter the function of soil microbial communities. This may be particularly important in terms of specialized roles per-
formed by Gram-negative bacteria, such as nitrification or CH4 oxidation, as our experiment demonstrated that elevated temperature increased the Firmicutes/Gram-negative bacteria ratio and increased indicators of stress in the Gram-negative bacterial community.

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