Mycorrhiza (2014) 24:581–593
DOI 10.1007/s00572-014-0577-4

ORIGINAL PAPER

Elevated CO_2 and O_3 effects on ectomycorrhizal fungal root tip communities in consideration of a post-agricultural soil nutrient gradient legacy

Carrie Andrew · Erik A. Lilleskov

Received: 18 January 2014 / Accepted: 25 March 2014 / Published online: 13 April 2014

Abstract Despite the critical role of EMF in nutrient and carbon (C) dynamics, combined effects of global atmospheric pollutants on ectomycorrhizal fungi (EMF) are unclear. Here, we present research on EMF root-level community responses to elevated CO_2 and O_3. We discovered that belowground EMF community richness and similarity were both negatively affected by CO_2 and O_3, but the effects of CO_2 and O_3 on EMF communities were contingent on a site soil pH and cation availability gradient. These results contrast with our previous work showing a strong direct effect of CO_2 and O_3 on sporocarp community dynamics and production. We discuss the possible role of carbon demand and allocation by EMF taxa in the discrepancy of these results. EMF communities were structured by a legacy of spatially defined soil properties, changing atmospheric chemistry and temporal dynamics. It is therefore necessary to understand global change impacts across multiple environmental gradients and spatio-temporal scales.

Keywords CO_2 · Carbon demand · Ectomycorrhizal fungi · O_3 · Legacies · pH · Root tips

Electronic supplementary material The online version of this article (doi:10.1007/s00572-014-0577-4) contains supplementary material, which is available to authorized users.

C. Andrew
Forest Resources and Environmental Sciences, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA

C. Andrew (✉)
Section for Genetics and Evolutionary Biology (EVOGENE), University of Oslo, Blindernveien 31, 0316 Oslo, Norway
e-mail: carrie.j.andrew@gmail.com

E. A. Lilleskov
US Forest Service, Northern Research Station, Forestry Sciences Laboratory, 410 MacInnes Drive, Houghton, MI 49931, USA

Introduction

Atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) concentrations directly affect plant carbon (C) fixation. This cascades through trophic levels, affecting growth and reproduction of heterotrophic organisms. Ectomycorrhizal fungi (EMF) might be especially sensitive to changes in primary production from altered CO_2 and O_3 concentrations given their reliance on current photosynthates (Högberg et al. 2001). CO_2- and O_3-mediated changes in host growth also indirectly affect soil properties (e.g., N, P, etc.), which in turn modify nutrient uptake by host trees, potentially mediated by, and feeding back to, mycorrhizal fungi (e.g., Andersen 2003; Finzi et al. 2007).

Despite the potential sensitivity of EMF to changing CO_2 and O_3 and their importance in C and nutrient cycling, we still know relatively little about how changes in these gasses alter EMF communities under field conditions. Major research has been conducted investigating aspects of EMF response to CO_2 or O_3. Several studies have focused on the effects of CO_2, though fewer have examined the effect of O_3, and even fewer have been able to look at full EMF communities (Online Resource 1). There are two studies to our knowledge which utilized large scale Free-Air CO_2 Enrichment (FACE) technology to examine community responses: the belowground work conducted at the Duke FACE site (Parrent et al. 2006; Parrent and Vilgalys 2007) and the aboveground work at the Aspen FACE site (Andrew and Lilleskov 2009). Only the latter explored the combined effects of CO_2 and O_3 on EMF communities.

At Duke FACE, Parrent et al. (2006) found an effect of CO_2 enrichment on the relative abundance of certain mycorrhizal taxa. Overall community richness and diversity were not affected by CO_2 level, but were by inorganic nitrogen availability. Soil resources are known to be strong drivers of ectomycorrhizal fungal community organization. High soil

N availability can lead to a complete shift in community dominants (Lilleskov 2005; Lilleskov et al. 2011; and references therein), and even subtle changes in N concentration can rapidly affect species richness (Avis et al. 2008). Soil pH and base cation availability can likewise affect ectomycorrhizal communities (e.g., Taylor and Finlay 2003; Kjøller and Clemmensen 2009; Rineau and Garbaye 2009). Variability in these soil properties could have confounding effects on EMF community analyses if not taken into account (Parrent et al. 2006; Lilleskov and Parrent 2007). Further, given the potential for CO_2 and O_3 to alter soil properties, it is important to understand whether FACE treatments affected soil nutrients with indirect effects on EMF communities.

In earlier work, we documented a strong response of EMF sporocarp communities to CO_2 and O_3 (Andrew and Lilleskov 2009) at the Aspen FACE site. This was in line with other fungal research conducted at the same site (Chung et al. 2006; Edwards and Zak 2011). The EMF sporocarp response abated over time, possibly from a convergence in community composition as forest stands reached equivalent ontogeny, combined with a shift towards clones better adapted to the treatments (Rey and Jarvis 1997; Staddon and Fitter 1998; Gorissen and Kuyper 2000; Pregitzer et al. 2006; Kubiske et al. 2007; Andrew and Lilleskov 2009). Here, we report on the belowground EMF community response to elevated CO_2 and O_3. We directly compare the results presented here with our earlier aboveground work, providing a comprehensive, multilevel overview of EMF response to CO_2 and O_3.

Given the previously documented responses of EMF sporocarp communities, our first objective was to quantify EMF root tip community response to elevated CO_2 and O_3. We expected that belowground EMF communities would respond similarly to sporocarps, with shifts in composition, dominance, or diversity. We then explored the effects of soil nutrients and spatial configuration on EMF communities while accounting for changes in CO_2 and O_3. Our results suggested a site–gradient response in EMF community structure that we hypothesized was mainly due to grading soil nutrient properties. In order to investigate these hypotheses, we characterized EMF root tip communities utilizing the standard FACE experimental design to address CO_2 and O_3 effects as well as further approaches to address nutrient gradient effects.

Materials and methods

Study area

Dickson et al. (2000) provide a complete description of the Aspen FACE site (45° 40′ 48″ N, 89° 37′ 48″ W). It has a cool continental climate with an average summer temperature of 18.3 °C. Precipitation averages 106.7 mm per month during the peak months of July through September. Treatment plots contained three sections of northern temperate deciduous trees. The present study was focused on half of these plot areas planted solely with *Populus tremuloides*. CO_2 and O_3 treatments were fixed at ambient (360–380 ppm for CO_2, 33–67 ppb for O_3) and elevated conditions (560–580 ppm for CO_2; 50–100 ppb for O_3) (Table 1).

The experiment was designed as a full factorial randomized complete block design. The blocking pattern captured variation in soil properties across the site along a north–south gradient (Fig. 1; Online Resource 2). There is no indication whether the gradient was a natural relict of geological history or influenced by site-specific patterns in land use and management. However, we have found no evidence of a management cause for the gradient. Historically, a potato and grains farm until purchase in 1972 by the US Forest Service, from 1976 to 1990 most of the site was planted with short-rotation hybrid larch and poplar clones. The remaining portions were old field. There was about 15 cm of sandy loam topsoil followed by a plow layer of clay accumulation 30 cm thick, with deeper clay layers found mostly on the northern portion of the site (Dickson et al. 2000). Investigators failed to find a significant difference in soil properties by replicate or gradient of the site, though percent C and N were slightly different between treatments towards the start of the experiment. All aspen and larch trees were removed from the site prior to experimental initiation. Trees were planted into a rye cover crop in 1997.

Roots–field sampling, morphotype, and biomass calculation

Five cores (15 cm diameter, 20 cm depth) in each plot were extracted in June 23 and July 02, 2003 and June 16, 2006 with 10 % (*c*. 50 g) used for this study. Soil was gently rinsed off the roots over a 1-mm sieve and frozen. Thawed roots were sorted in 95 % ethanol to avoid DNA degradation. Within each core, all ectomycorrhizal root tips were sorted into putative morphotypes based on the criteria of Agerer (2002). Freeze-dried tips were weighed, and biomass per unit soil volume was calculated using the core volume and root dry mass.

Molecular analysis

In 2003, DNA was extracted from one to two representative root tips of each morphotype from each core using the CTAB mini-prep procedure (Gardes and Bruns 1993) and PCR-amplified with the fungal-specific primer ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). An initial denaturation phase of 75 s at 94.5 °C was followed by 35 cycles of 95 °C for 35 s, 55 °C for 55 s, 72 °C for 42 s (+ 6 s per cycle), followed by one 10-min final extension at 72 °C. Average ectomycorrhizal root tip biomass ranged from 0.11 to 0.28 mg/m^3 in 2003.

Table 1 Carbon demand classifications for taxa present at the Aspen FACE site (independent of treatments). Taxa are sorted alphabetically within each category

Taxon	Average sporocarp biomass (mg/cm^2)	Average root tip biomass per core (mg/cm^3)	Ratio of sporocarp to root tip biomass	Ln (ratio of sporocarp to root tip biomass)	Sporocarp to root tip rank[a]	Exploration type	Exploration type rank[b]	Carbon demand rank[c,d]	Carbon demand classification
Galactinia sp.	–	0.00838	–	–	2.5	Contact	1	6.5	Low
Tuber/*Helvella* group	–	0.01258	–	–	2.5	Contact	1	6.5	Low
Tuber (genus level)	–	0.33333	–	–	2.5	Short distance or contact	1.5	8.5	Low
Peziza c.f. *badia*	0.0003	0.01062	0.03	−3.44	1	Short distance	2	9	Low–medium
Inocybe curvipes/sororia group	0.0024	0.00528	0.45	−0.80	2	Short distance	2	10	Low–medium
Peziza c.f. *sylvestris*	0.0005	0.00143	0.38	−0.97	2	Short distance	2	10	Low–medium
Geopora (genus level)	–	0.05556	–	–	2.5	Short distance	2	10.5	Low–medium
Hydnobolites sp.	–	0.00679	–	–	2.5	Short distance	2	10.5	Low–medium
Meliniomyces sp.	–	0.00132	–	–	2.5	Short distance	2	10.5	Low–medium
Pachyphloeus/Amylascus group	–	0.00604	–	–	2.5	Short distance	2	10.5	Low–medium
Peziza/Terfezia group	–	0.01184	–	–	2.5	Short distance	2	10.5	Low–medium
Sebacina (*Serendipita*) sp.	–	0.00298	–	–	2.5	Probably short distance	2	10.5	Low–medium
Sphaerosporella sp.	–	0.00197	–	–	2.5	Short distance	2	10.5	Low–medium
Terfezia/Peziza depressa group	–	0.00351	–	–	2.5	Short distance	2	10.5	Low–medium
Tomentella (genus level)	–	0.32222	–	–	2.5	Contact, short or medium distance (smooth subtype)	2	10.5	Low–medium
Inocybe flocculosa	0.0222	0.00632	3.52	1.26	3	Short distance	2	11	Medium
Inocybe lacera	0.0074	0.00775	0.96	−0.04	3	Short distance	2	11	Medium
Hebeloma mesophaeum group	0.0074	0.02457	0.30	−1.20	2	Medium distance subtype fringe or short distance	2.5	12	Medium
Russula (genus level)	0.0004	0.00046	0.84	−0.18	2	Short or medium distance smooth	2.5	12	Medium
Cortinarius obtusus group	0.0009	0.00665	0.14	−1.96	1	Medium distance fringe subtype	3	13	Medium
Laccaria tortilis	0.0004	0.00433	0.10	−2.33	1	Medium distance smooth subtype or fringe subtype	3	13	Medium
Hebeloma crustuliniforme group	0.0094	0.00171	5.51	1.71	4	Medium distance subtype fringe or short distance	2.5	14	Medium
Laccaria laccata	0.0025	0.00441	0.56	−0.59	2	Medium distance smooth subtype or fringe subtype	3	14	Medium
Thelephora terrestris	0.0020	0.00300	0.66	−0.41	2		3	14	Medium

Table 1 (continued)

Taxon	Average sporocarp biomass (mg/cm^2)	Average root tip biomass per core (mg/cm^3)	Ratio of sporocarp to root tip biomass	Ln (ratio of sporocarp to root tip biomass)	Sporocarp to root tip rank[a]	Exploration type	Exploration type rank[b]	Carbon demand rank[c,d]	Carbon demand classification
Cortinarius acutus group	0.0003	0.00010	3.43	1.23	3	Medium distance, smooth subtype Medium distance fringe subtype	3	15	Medium–high
Cortinarius bulbosus group	0.0518	0.01134	4.57	1.52	3	Medium distance fringe subtype	3	15	Medium–high
Amanita muscaria	0.0790	0.01071	7.38	2.00	4	Medium distance smooth subtype	3	16	Medium–high
Leccinum insigne	0.2592	0.01029	25.18	3.23	4	Long distance	4	20	High
Paxillus involutus	0.0162	0.00112	14.49	2.67	4	Long distance	4	20	High

Data sources: Andrew and Lilleskov (2009); Agerer (2001); Agerer (2006); Hobbie and Agerer (2010); Tedersoo et al. 2006; L. Tedersoo, personal communication

Low 5–8, *Low–medium* 9–10, *Medium* 11–14, *Medium–high* 15–16, *High* 17–20

[a] Rank was based on partitioning the natural log of sporocarp:root tip biomass into quartiles

[b] Contact=1, Short distance=2, Medium distance=3, Long distance=4

[c] When more than one exploration type was reported, the average rank was reported

[d] Carbon-demand rank (CD) was calculated as CD=4(E)+S:R. Sporocarp carbon demand (per unit root tip) used the ratio of sporocarp to mycorrhizal root tip biomass (S:R), where sporocarp biomass values were from Andrew and Lilleskov (2009) and root tip biomass from this study (refer to methods for more information). Exploration types (E) were ranked for relative carbon demand, assuming longer distance exploration types have higher carbon demand. The weighting of E was based on an average sporocarp biomass of c. 25 % compared to hyphal biomass in the study system (Andrew and Lilleskov 2009; Andrew and Lilleskov, unpublished). OTUs lacking sporocarp data (hypogeous and crustose sporocarps) received an S:R rank of 2.5. This balanced error in over- or underestimation of the true rank and, given the greater weighting of hyphal biomass, changing this value had minimal effect on the CD value

Fig. 1 A map of the Aspen-FACE site landscape, near Rhinelander, WI, USA. The treatments within each block are provided along with the soil nutrient gradient denoted as grading across the site with the *dotted white line*. Refer to Online Resource 2 and Dickson et al. (2000) for soil property values of each treatment–plot combination. Photograph credit: David F. Karnosky

The 2003 morphotypes were grouped through restriction fragment length polymorphism (RFLP) and terminal restriction fragment length polymorphism (TRFLP). Single-banded PCR products were identified via gel electrophoresis and included in further analysis. RFLP was performed with HinFI and DpnII (New England Biolabs, Ipswich, MA). Restriction patterns were visualized with 1 %/2 % gel agarose and imaged with the Kodak EDAS 290. Fragment sizes were estimated using the Kodak 1D software (both from Eastman Kodak Company, Rochester, NY). Morphotypes were grouped into coarse resolution RFLP groups and analyzed with TRFLP using the Ceq 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). HinFI and HaeIII (New England Biolabs, Ipswich, MA) were used for TRFLP analysis with the primers ITS1F and ITS4 labeled with WellRED fluorescent dyes D3 and D4, respectively (Proligo, Boulder, CO). Amplified DNA of one TRFLP group member was cleaned with the QIAquick PCR Purification Kit (Qiagen, Valencia, CA) and sequenced.

To increase process efficiency, representatives of all 2006 EMF morphotypes from each core were directly sequenced. Fungal DNA was extracted with the REDExtract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, MO) with volume modified to 10 % (Avis et al. 2008). Amplification and proofing of PCR products followed the parameters above. Average biomass of ectomycorrhizal root tips ranged from 0.06 to 0.10 mg/m^3 in 2006.

Samples were sequenced at the Nevada Genomics Center, University of Nevada-Reno on an ABI3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Double-stranded DNA sequencing of each TRFLP type was used for the 2003 samples. Based on those results, single-stranded sequencing using ITS4 was used for each sample of the 2006 data set. The consistency of morphotype designations was assured by periodic sequencing of more than one tip within a given group.

Post-sequencing analysis—2003 and 2006 samples

Operational taxonomic units (OTUs) were designated based on BLAST searches in GenBank (NCBI) and UNITE (Kõljalg et al. 2005) databases (Online Resource 3). Sequences matching vouchers with ≥98 % similarity across at least 450 base pairs were considered the same OTU. Sequences with greater than 95 % similarity were considered the same genus; those with at least 90 % similarity were considered the same family (criteria of Landeweert et al. 2003). Sequences equally matching multiple species were either placed within a broader species group or left without a specific epithet. Samples were grouped together when restriction enzyme digestion did not discriminate between sequence OTUs (Online Resource 4). Samples were additionally lumped based on the sporocarp taxonomic resolution, which included species groups for certain taxa. A representative sequence from each OTU was submitted to GenBank (accession numbers JX316771-JX316829).

Soil nutrient analysis

Ten percent of each 2006 soil core and two additional sets collected in May 23–26, 2008 and September 16–20, 2008 was analyzed for nutrient content. Percent carbon and nitrogen

values were determined on a Fisons NA 1500 Elemental Analyzer (ThermoQuest Italia, Milan, Italy) at Michigan Technological University (Houghton, Michigan). Subsamples were analyzed for pH, extractable phosphorus (Bray P1 method), exchangeable cations (sodium acetate method), nitrate, and ammonium (Colorimetric, KCl method) at Michigan State University (East Lansing, MI).

Data analyses

Analyses were conducted mainly in R versions 2.7.1 to 2.14.0 (R Development Core Team 2006; packages ade4, FactoMineR, labdsv, MASS and vegan) along with SPSS version 13.0 (SPSS Inc., Chicago, IL). Univariate-dependent variables (richness and similarity) were analyzed with repeated measures ANOVA, with the independent factors CO_2, O_3, block, and the interaction of CO_2 and O_3. To test for treatment effects on successional dynamics, taxa were categorized into one of two broad successional groups (earlier or later; Online resource 5). We understand that this description simplifies successional patterns (e.g., Twieg et al. 2007), but we believe that these two categories still provide insight into fungal community dynamics and further division is unwarranted given the available information.

Shared species indices were computed in EstimateS version 8.0 (Colwell 2006). Within treatment-shared species, similarity was computed using the Chao-Sorenson abundance-based estimator, correcting the Sorenson index for unseen species due to low sample size and/or high diversity (Chao et al. 2005).

Community response was analyzed using relative biomass and Bray-Curtis distance using PERMANOVA. The fixed factors CO_2 and O_3 were analyzed with the core date included as a fixed factor. The data were also analyzed with block as a fixed factor, utilizing sampling year for replicates. Post hoc univariate tests for individual taxa were performed only with significant factors from the multivariate analysis, thus protecting for experiment-wise error of multiple comparisons. Non-metric multidimensional scaling (NMDS) biplots were created from the distance matrices.

A MANOVA assessed the overall significance of the study design on soil properties at the site after standardizing by the site average for each year. An average of the 2006 and 2008 values (Online Resource 1) was used as predictors for the EMF communities. Soil and environmental variables were analyzed in conjunction with the community data using with the ordicomp and envfit functions. Soil properties were regressed against Hellinger-transformed species community data, while controlling for potential spatial correlates, using a partial redundancy analysis (RDA) and variance partitioning on the adjusted R^2 values (Borcard et al. 2011; Legendre and Legendre 2012). Forward selection using the ordistep function along with calculation of variance inflation factors (VIF) confirmed variable inclusion in the model. The RDA and variance partitioning approach are preferable to partial mantel tests when the objective is to ascertain environmental effects on community data in consideration of spatial correlation (Legendre and Legendre 2012). It provides an assessment of how much species variation can be attributed to edaphic environmental variables when the effect of space is removed (Borcard et al. 2011).

Taxon-specific correlations with spatial structure and the site soil nutrient gradient were analyzed with partial mantel tests. Regression analysis was run at the taxon level after verifying a lack of spatial correlation. Taxon-specific relative abundance was averaged across 2 years and regressed against soil properties in SigmaPlot version 9.01 (Systat Software, Inc.) for taxa present in 50 % or more of the plots. Non-linear Gaussian or exponential decay regression fits accounted for non-linearities and only applied if tests for normality and constant variance were met.

Results

EMF root tip community response to CO_2 and O_3

Within-treatment ectomycorrhizal fungal community similarity was highest with ambient conditions in both years (Fig. 2a). An O_3 effect depended on the CO_2 treatment ($CO_2 \times O_3$ $P=0.045$) with a strong divergence between ambient and elevated O_3 only under ambient CO_2 conditions. Under elevated O_3 with ambient CO_2, there was marginally less shared species similarity than under ambient O_3 (O_3 $P=0.058$). Shared species similarity was comparable with elevated CO_2 regardless of O_3 level.

Taxon richness was also the highest under ambient conditions. The O_3 effect depended marginally on CO_2 treatment ($CO_2 \times O_3$ $P=0.076$; Fig. 2b). Under ambient O_3, richness was lower with elevated CO_2 than with ambient CO_2 (CO_2 $P=0.027$) while under elevated O_3 richness was not affected by CO_2 level. Richness was strongly affected by the site blocking structure ($P=0.032$). It decreased, regardless of CO_2 and O_3 levels, from one end of the site to the other ($R^2=0.370$).

Community composition was marginally affected by CO_2 level ($P=0.078$) and more strongly by a $CO_2 \times O_3 \times$ block interaction ($P=0.033$) and block ($P=0.008$; Table 2, Fig. 1). Composition within the elevated CO_2 treatments was significantly different between the most distal portions of the site (along a north–south transect; $P=0.010$; Fig. 3).

Individual taxon and successional responses to CO_2 and O_3

Several taxa exhibited significant or marginal responses to elevated CO_2 or O_3 (Online Resources 5 and 6). Elevated CO_2 decreased the relative biomass of *Tomentella* sp. 'A'

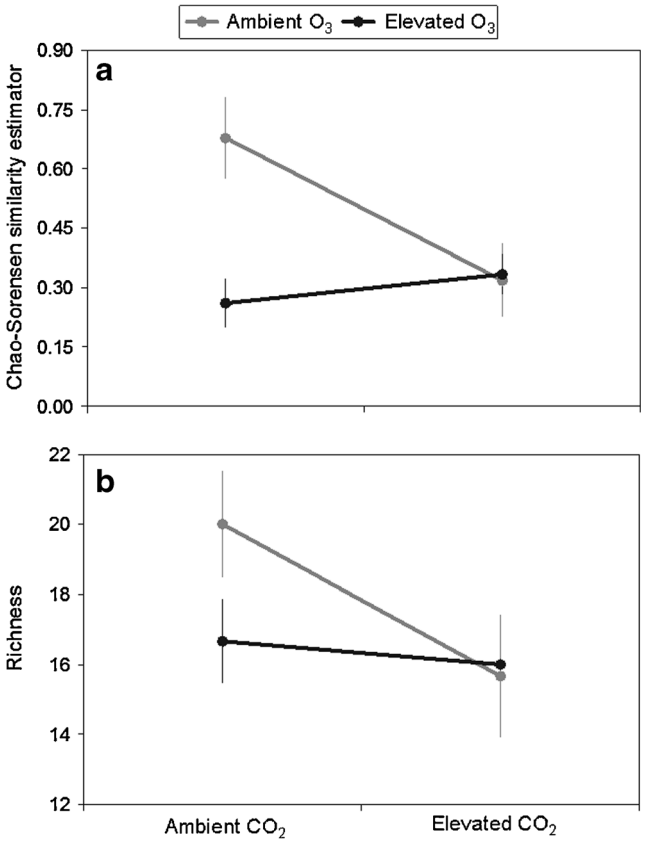

Fig. 2 Interaction plots of within-treatment similarity and richness of ectomycorrhiza communities exposed to elevated CO_2 and/or O_3 at the Aspen FACE site. CO_2 level is reported on the *x*-axis while ambient O_3 is *gray* and elevated O_3 is *black*. **a** Similarities were averaged across the years. A larger number indicates greater within-treatment species similarity. **b** Total pooled OTU richness of species for 2003 and 2006. Standard *error bars* (±) are provided. Please note that lines are drawn to connect treatments and do no connote a linear relationship between points

regardless of O_3 level (P=0.036). *Tomentella* sp. 'C', the only OTU present in all 12 plots with pooled sample dates, had less average relative biomass under elevated CO_2 for both years and greater relative biomass under elevated O_3 in 2003 and under elevated O_3 with elevated CO_2 in 2006 (year×CO_2, P=0.049; year×CO_2×O_3, P=0.038). *Sebacina* (=*Serendipita*) sp. had marginally greater relative biomass under elevated O_3 with ambient CO_2 (P=0.090). The *Cortinarius obtusus* group had marginally greater relative biomass under elevated CO_2, especially with elevated O_3 (CO_2 P=0.096). EMF root tip biomass of earlier successional taxa (Online Resource 5) decreased from 2003 to 2006 (Online Resource 7; P=0.023), but there were no treatment effects on successional trajectory.

Soil properties

Soil properties were spatially structured across the site (block P=0.032) with the following significantly different between site blocks: pH (P=0.017), P (P=0.021), Ca^{2+} (P=0.004), and Mg^{2+} (P=0.003). These differences were independent of CO_2 (P=0.144) and O_3 (P=0.221) levels. Plot to plot differences in soil properties were stable both with and between years, and the relative ranks by block remained stable across time (Fig. 4).

Many soil properties (averaged 2006 and 2008) were correlated with one another. Specifically, in pairwise comparisons, pH, Ca^{2+}, and Mg^{2+} were positively correlated (Ca^{2+} and Mg^{2+} r=0.99, Ca^{2+} and pH r=0.90, Mg^{2+} and pH r=0.94). Distance between sample points was a significant predictor of those soil chemical variables (Mantel's r=−0.77, −0.84, and −0.85 respectively). Phosphorus and pH were also weakly positively correlated (r=0.61).

EMF root tip community in relation to spatial structure and soil chemistry

Correlations between the ectomycorrhizal communities and soil properties were significant for pH (P=0.001, r^2=0.63), Mg^{2+} (P=0.001, r^2=0.59), block (P=0.001, r^2=0.54), Ca^{2+} (P=0.003, r^2=0.52), year (P=0.019, r^2=0.34), and marginally P (P=0.070, r^2=0.22) (Fig. 3). A significant correlation existed with the community composition and environment covariates when controlling for spatial patterning in the RDA analysis (P=0.004). The optimal model for RDA and variance partitioning included pH as the only significant environmental variable due to the high degree of correlation of pH with Ca^{2+} and Mg^{2+}. Spatial variation alone failed to comprise a significant portion of the partitioned variance, although 4.6 % variance was shared between environmental and spatial matrices, and 4.1 % variance attributed to environment alone. We interpret this to mean that spatial configuration alone failed to affect EMF community composition while soil environmental variables (i.e., pH and correlates) and the spatial patterning of said environmental variables did significantly affect EMF community structure.

Individual taxon responses to spatial structure and soil chemistry

We found that soil nutrient qualities strongly influenced mycorrhizal OTU abundance. Three genera exhibited a clear cation/pH response. Root tip relative abundance of the genus *Inocybe* was negatively related to Mg^{2+} (r^2=0.63, P=0.001) and Ca^{2+} levels (r^2=0.68, P=0.002) due largely to the effect of *Inocybe lacera* (Fig. 5). Similarly, *Tuber* species abundance was negatively related to Mg^{2+} (r^2=0.79, P=0.0004), Ca^{2+} concentrations (r^2=0.75, P=0.0008), and soil pH (r^2=0.48, P=0.008) largely driven by *Tuber* sp. 'A' (Fig. 5). In contrast, the genus *Hebeloma* increased in relative abundance with increased Mg^{2+} (r^2=0.90, P<0.0001), pH (r^2=0.89, P<0.0001), and Ca^{2+} (r^2=0.94, P<0.0001), this time due largely to the response by the *Hebeloma mesophaeum* group (Fig. 5). Direct taxon-level spatial effects were weak:

Table 2 PERMANOVA tables for effects of CO_2 and O_3 treatments on ectomycorrhizal root tip community composition

	Degrees of freedom	Sum of squares	Mean square	F	*P* value
Analysis of CO_2, O_3, and sampling year as fixed factors					
CO_2	1	4,603.45	4,603.45	1.3606	0.206
O_3	1	4,223.51	4,223.51	1.2483	0.256
Year	1	4,998.22	4,998.22	1.4773	0.163
$CO_2 \times O_3$	1	1,994.33	1,994.33	0.5894	0.812
$CO_2 \times$ Year	1	2,251.48	2,251.48	0.6654	0.741
$O_3 \times$ Year	1	1,156.49	1,156.49	0.3418	0.963
$CO_2 \times O_3 \times$ Year	1	1,424.38	1,424.38	0.4210	0.925
Residual	16	54,135.02	3,383.44		
Total	23	74,786.89			
Analysis of CO_2, O_3, and block as fixed factors					
CO_2	1	4,603.45	4,603.45	1.7979	0.078
O_3	1	4,223.51	4,223.51	1.6495	0.111
Block	2	11,267.27	5,633.64	2.2002	0.008
$CO_2 \times O_3$	1	1,994.33	1,994.33	0.7789	0.625
$CO_2 \times$ Block	2	7,088.21	3,544.11	1.3842	0.154
$O_3 \times$ Block	2	5,518.97	2,759.48	1.0777	0.380
$CO_2 \times O_3 \times$ Block	2	9,365.42	4,682.71	1.8288	0.033
Residual	12	30,725.73	2,560.48		
Total	23	74,786.89			

Tomentella sp. 'B' was the only taxon with a statistically significant partial mantel correlation indicative of spatial structure (P=0.027).

Overall comparison of EMF root tips and sporocarps

Five taxa were found as both mycorrhizas and sporocarps in 50 % or more of the plots with sampling years pooled. In descending order of frequency, these were *Hebeloma crustuliniforme* group, *C. obtusus* group, *I. lacera*, *Leccinum insigne*, and *Cortinarius bulbosus* group. Other taxa found in fewer plots included (in descending order): *Hebeloma mesophaeum* group, *Laccaria tortilis*, *Peziza* c.f. *badia* Pers./*P.* sp. RH1314, *Amanita muscaria*, *Paxillus involutus*, and *Thelephora terrestris*. Five taxa were found only aboveground, 28 were observed only belowground, and four were found as both mycorrhizas and sporocarps, but not in the same plot (Online Resource 5).

L. insigne, which dominated sporocarp production under elevated CO_2 (regardless of O_3 level) in 2003, had become dominant in all treatments by 2006 (Andrew and Lilleskov 2009; Online Resource 5). In contrast, *L. insigne* remained uncharacterized belowground in 2003, but was found in 2006 with no significant treatment effect. *L. insigne* was found belowground in eight plots while it produced sporocarps in all 12 plots.

Discussion

The effects of elevated CO_2 and O_3 on species similarity, taxonomic richness, and community structure support their importance in ectomycorrhizal fungal community structuring. The effects on similarity and richness were largely similar even though CO_2 stimulates while O_3 depresses photosynthetic C gain. Nutrient availability was highly important in determining belowground mycorrhizal community structure, with important interactions between CO_2 and O_3 levels.

When the present study is compared to our previous study of sporocarp communities (Andrew and Lilleskov 2009), CO_2 and O_3 concentrations more strongly influenced production and composition of EMF sporocarps than ectomycorrhizal root tips. This is further supported by mycelial production responses, which were not significant (Andrew et al. 2014). This aboveground–belowground discrepancy in CO_2 and O_3 response suggests that sporocarps are most sensitive to a reduction in C supply. The greater sensitivity of sporocarp community response than ectomycorrhizal root tip response has also been found in response to nitrogen deposition (Wallenda and Kottke 1998; Lilleskov 2005). Climate change has likewise been attributed to temporal patterns in sporocarp production and fruiting (Büntgen et al. 2011; Kauserud et al. 2012; Boddy et al. 2014), again illustrating the strong response of sporocarps to global change components. One thing not yet quantified in these studies is how future fungal assemblages may be affected over the long term by dispersal-

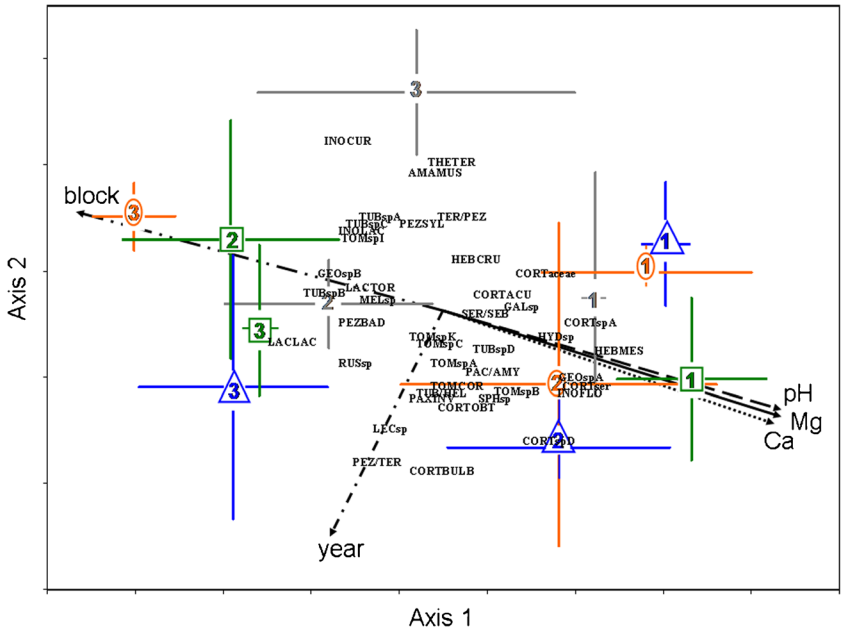

Fig. 3 NMDS biplot of ectomycorrhiza community composition, averaged across sample years, at the Aspen FACE site. *Numbers* correspond to the spatial block structure. *Gray open numbers* are ambient plots, *orange ovals* are elevated CO_2, *blue triangles* are elevated O_3, and *green squares* are elevated CO_2+O_3. Soil properties are overlaid as vectors with only statistically significant variables shown. Standard *error bars* (±) show variability within communities. Taxon code: *Amanita muscaria* (AMAMUS), Cortinariaceae (Hymenogastraceae) sp. (CORTaceae), *Cortinarius acutus* group (CORTACU), *Cortinarius bulbosus* group (CORTBULB), *Cortinarius obtusus* group (CORTOBT), *Cortinarius* sp. subgenus Sericeocybe (CORTser), *Cortinarius* sp. 'A' (CORTspA), *Cortinarius* sp. 'D' (CORTspD), *Galactinia* sp. (GALsp), *Geopora* sp. 'A'/*G. cervina* (GEOspA), *Geopora* sp. 'B' (GEOspB), *Hebeloma crustuliniforme* group (HEBCRU), *Hebeloma mesophaeum* group (HEBMES), *Hydnobolites* sp. (HYDsp), *Inocybe curvipes*/*sororia group* (INOCUR), *Inocybe flocculosa* (INOFLO), *Inocybe lacera* (INOLAC), *Laccaria laccata* (LACLAC), *Laccaria tortilis* (LACTOR), *Leccinum* sp. (LECsp), *Meliniomyces* sp. (MELsp), *Pachyphloeus thysellii*/*Amylascus* group (PAC/AMY), *Paxillus involutus* (PAXINV), *Peziza* sp. '*B*'/*Terfezia* group (PEZ/TER), *Peziza* c.f. *badia*/*P.* sp. RH1314 (PEZBAD), *Peziza* c.f. *sylvestris*/*Pachyphlodes* group (PEZSYL), *Russula* sp. (RUSsp), *Sebacina* (*Serendipita*) sp. (SER/SEB), *Sphaerosporella* sp. (SPHsp), *Terfezia*/*Peziza depressa* group (TER/PEZ), *Thelephora terrestris* (THETER), *Tomentella coerulea* (TOMCOR), *Tomentella* sp. 'A' (TOMspA), *Tomentella* sp. 'B' (TOMspB), *Tomentella* sp. 'C' (TOMspC), *Tomentella* sp. 'I' (TOMspI), *Tomentella* sp. 'K' (TOMspK), *Tuber*/*Helvella* group (TUB/HEL), *Tuber* sp. 'A' (TUBspA), *Tuber* sp. 'B' (TUBspB), *Tuber* sp. 'C' (TUBspC), *Tuber* sp. 'D' (TUBspD)

mediated changes in sporocarp composition and production (e.g., Peay et al. 2007).

It is informative to examine taxon-level C demand of those that responded to the treatments. *L. insigne*, a sporocarp dominant in 2003–2006 (Andrew and Lilleskov 2009), had an infrequent and clumped distribution on root tips similar to other boletes (e.g., Gardes and Bruns 1996; Rosling et al. 2003; Lilleskov et al. 2004; Online Resource 5). Low percent root colonization is contrasted with high-sporocarp production and combined with highly differentiated long distance rhizomorphs (Agerer 2006; Hobbie and Agerer 2010), all working to classify *L. insigne* as a high-carbon demand fungus (Table 1). Positive sporocarp responses of *L. insigne* to elevated CO_2 (Andrew and Lilleskov 2009) suggest this high-carbon demand fungus was able to acquire more carbon under elevated CO_2. However, here, we found no treatment effect on its root tip abundance. While it is possible that sampling was insufficient to properly quantify root tip abundance, given its life history characteristics, it is just as likely that carbon was allocated preferentially to reproduction rather than root tips. We expect taxa with similar traits to respond more strongly to CO_2 levels than other taxa, which invest less C into reproduction, but suggest that this may only be quantifiable in terms of sporocarp productivity.

The *C. obtusus* group also increased in relative abundance under elevated CO_2. This fungal group has a relatively high C demand, partly due to its medium distance fringe exploration type (Table 1). The response documented here is consistent with negative effects of girdling on the Cortinariaceae in a northern Swedish forest experiment (Yarwood et al. 2009). In a different study, root excision likewise decreased the prevalence of *Cortinarius* mycorrhizas after 5 days (Lindahl et al. 2010). This all suggests that *Cortinarius* is strongly tied to current photosynthate, although exceptions to this pattern can occur (e.g., Fransson 2012 and references therein).

Elevated O_3 favored four taxa, increasing their relative abundances: *Tomentella* sp. 'A', *Tomentella* sp. 'C', *Sebacina* (*Serendipita*) sp., and the *H. crustuliniforme* species group. These taxa seem to withstand conditions with potentially limited current photosynthate. *Tomentella lapida* is unaffected by girdling (Pena et al. 2010), suggesting that this congener of *Tomentella* spp. 'A' and 'C' is tolerant of reduced C supply. *Tomentella sublilacina* was also one of two dominants on

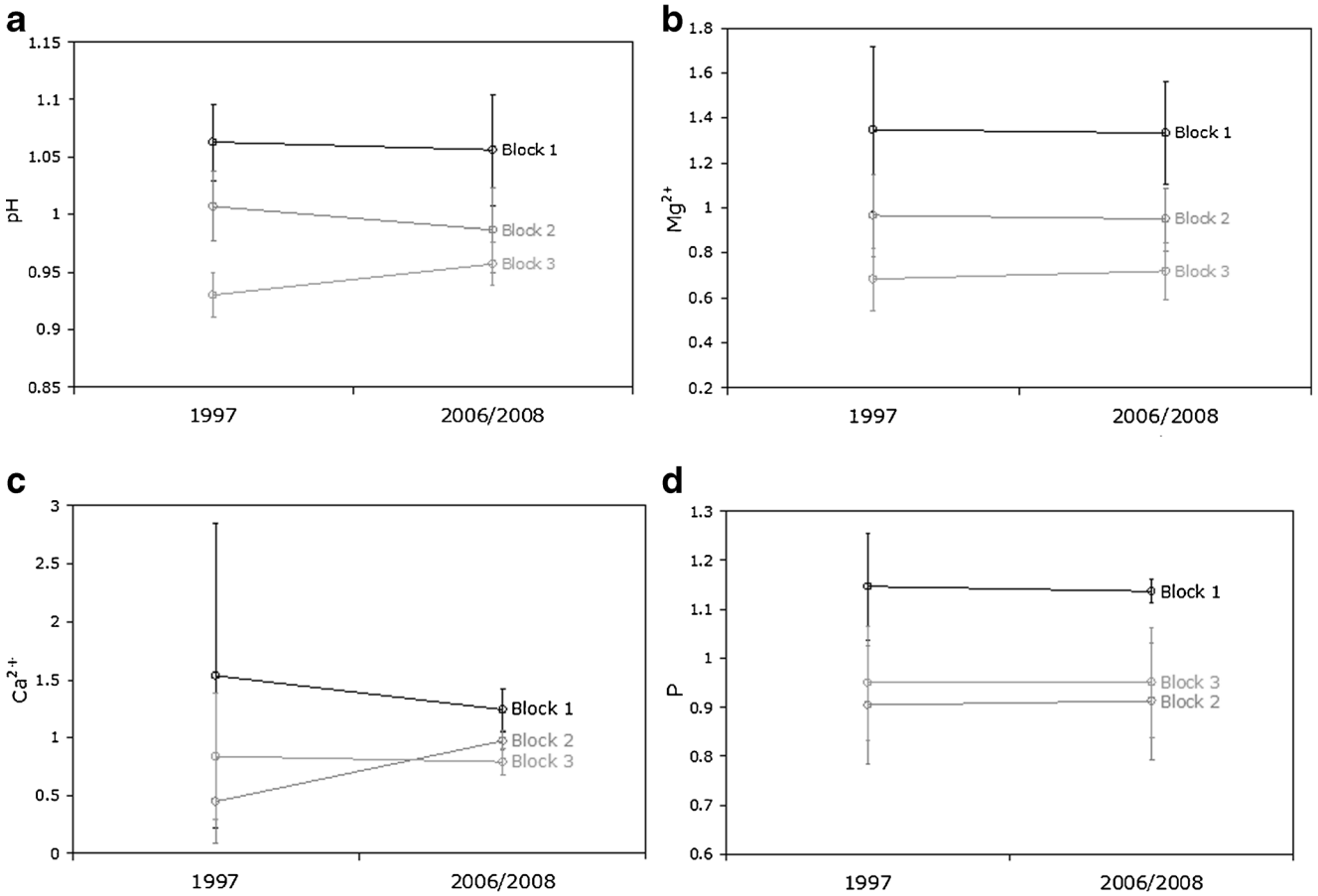

Fig. 4 Block effects on soil nutrient properties. The original 1997 values are compared to more current values (2006 and 2008) for **a** pH, **b** Mg^{2+}, **c** Ca^{2+}, and **d** P. Values provided are a unitless relativization by the average so that a value of one is equal to the site average. Lines connect individual blocks by year; this does not denote a simple linear trend from 1997 to 2008 but rather connects points for easier reference. Block 1 is shown in *black*, block 2 in *medium gray*, and block 3 in *light grey*. Standard *error bars* (±) provided

hemlock and yellow birch seedlings on nurse logs in densely shaded hemlock-dominated stands from a more northern Michigan site, suggesting an ability to colonize roots under conditions of low-carbohydrate availability (Poznanovic et al. submitted). Similarly, unhealthy trees can have a higher proportion of *Sebacina* and Thelephoraceae root tips (Nara et al. 2003). Whether this is indicative of low C supply from hosts is uncertain, but when combined with the responses here does suggest the possibility and justifies further investigation. In contrast, *H. crustuliniforme* is classified as a fairly high C demand fungus because of its medium distance exploration type and high-sporocarp productivity (Table 1). Consistent with this, it increased *Pseudotsuga menziesii* seedling nutrient concentrations while decreasing seedling size, indicative of high-sink strength for carbohydrates (Dosskey et al. 1990). It also responded to elevated CO_2 with dramatically elevated mycelial biomass (Fransson et al. 2005). Together, this suggests factors other than C demand that may have mediated the *H. crustuliniforme* treatment response.

The taxa previously mentioned exemplify the complexity of responses to CO_2 and O_3 levels. It follows that much of these differences may be due to carbon allocation, partitioning, and demand (Table 1). We suggest that critical investigations of fungi in light of these characteristics will clarify what can be seemingly nebulous results. The incorporation of root exudate estimates across varying EMF taxa, which likewise is affected by CO_2 concentration (Johansson et al. 2009), can only help illuminate these complex processes. Further, community-wide analyses at the root tip level may be insufficient to capture fungal responses to CO_2 and O_3 levels given the multitude of life history strategies possible (e.g., Hobbie and Agerer 2010).

The most important determinant of EMF root tip community structure was the spatially defined soil properties. A soil property's legacy as a major determinant in mycorrhizal fungal community dynamics is suggested since treatments failed to have a direct effect on soil properties. The strong nutrient effect is similar to the findings by Parrent et al. (2006), who found nitrogen availability more strongly predicted community structure than CO_2 level. The role of ectomycorrhizal fungi in nutrient and carbon cycling was found highly important for this system (Phillips et al. 2012). Nitrogen, however,

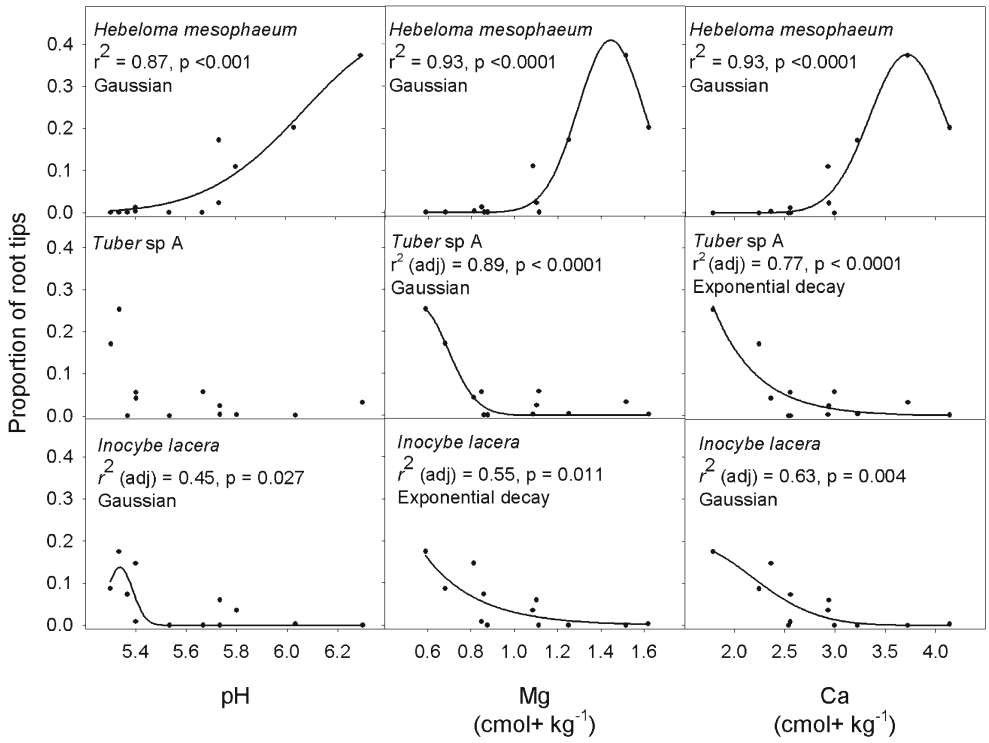

Fig. 5 Non-linear regression analyses of three dominant taxa at the Aspen-FACE site in response to three main site soil variables, with statistics reported for values of $P \leq 0.05$. Note that *Tuber* sp. A could not be regressed against soil pH with a Gaussian or exponential decay model. Genus-level responses strongly mirrored these taxon responses and are thus not shown here. See text for genus-level responses

cannot be exclusively implicated with the interactions of soil properties and EMF responses to changing CO_2. Shaw et al. (1992) found phosphorous concentration structured EMF on *Pinus sylvestris* more strongly than open-air O_3 or SO_2 treatments. In contrast, we found pH, Ca^{2+}, Mg^{2+}, and, less so, P as the most important predictors of community structure. Effects of CO_2 concentrations and N availabilities likewise interact contingent on life history traits of ectomycorrhizal taxa, such as nitrophilic or nitrophobic tendencies of fungi (Alberton and Kuyper 2009). N availability was uniformly low in this study system, so all else being equal, N would be expected to be less influential than the more variable cations, pH, and P.

Individual taxa differed strongly in their response to the pH–cation gradient. A positive relationship between pH and abundance of *Hebeloma* sporocarps has been observed previously (Kraepelin and Michaelis 1997; Lilleskov et al. 2001), but we are aware of no other belowground studies delineating this pattern. *I. lacera* has been found to be relatively tolerant of acid deposition in Europe, perhaps reflecting adaptation to low pH soils. The negative relationship between pH and *Tuber* abundance is somewhat surprising, as many *Tuber* species are associated with higher pH soils (Giovannetti et al. 1994). This could reflect species-specific differences in pH tolerance or the influence of unmeasured variables.

Conclusion

Ectomycorrhizal fungal communities were affected by a combination of atmospheric variables, site nutrient variability, and its associated spatial patterning. Plant nutrition and host C allocation to EMF interact in complex ways even in apparently uniform landscapes, leading to differing community responses across nutrient availability gradients, as evident in this study. Large changes in atmospheric chemistry affected EMF communities less at the root tip level compared to the sporocarp level. One major challenge in understanding EMF community response to global change is disentangling effects of atmospheric chemistry from those of other environmental factors.

Acknowledgments The authors thank Peter Avis, Rosanne Healy, Leho Tedersoo, and Bryant Scharenbroch for their nomenclatural assistance, advice, and/or suggestions. We appreciated the molecular work aided by Jessica Bibbee and Andy Quinn, as well as field and lab help from Joy Andrew, Robert Andrew, and Lynette Potvin. Funding was provided by the USDA Forest Service, Northern Research Station, two Graduate Research Grants awarded to C. Andrew from the Ecosystem Science Center (Michigan Technological University), and a Michigan Technological University Graduate School Finishing Fellowship Grant. We thank the US Department of Energy and the FACE Steering Committee for implementing and maintaining the Aspen FACE site for the duration of this study.

Conflict of interest The authors declare that they have no conflict of interest.

References

Agerer R (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Agerer R (2002) Colour atlas of ectomycorrhizae. Einhorn, Schwäbisch Gmünd

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Prog 5:67–107

Alberton O, Kuyper TW (2009) Ectomycorrhizal fungi associated with *Pinus sylvestris* seedlings respond differently to increased carbon and nitrogen availability: implications for ecosystem responses to global change. Glob Chang Biol 15:166–175

Andersen CP (2003) Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol 157:213–228

Andrew C, Lilleskov EA (2009) Productivity and community structure of ectomycorrhizal fungal sporocarps under increased atmospheric CO_2 and O_3. Ecol Lett 12:813–822

Andrew CJ, Van Diepen LTA, Miller RM, Lilleskov EA (2014) Aspen-associated mycorrhizal fungal production and respiration as a function of changing CO_2, O_3, and climatic variables. Fun Eco

Avis PG, Mueller GM, Lussenhop J (2008) Ectomycorrhizal fungal communities in two North American oak forests respond to nitrogen addition. New Phytol 179:472–483

Boddy L, Büntgen U, Egli S, Gange AC, Heegaard E, Kirk PM, Mohammad A, Kauserud H (2014) Climate variation effects on fungal fruiting. Fun Eco

Borcard D, Gillet F, Legendre P (2011) Numerical Ecology with R. Springer, New York

Büntgen U, Kauserud H, Egli S (2011) Linking climate variability to mushroom productivity and phenology. Front Ecol Environ 10:14–19

Chao A, Chazdon RL, Colwell RK, Shen TJ (2005) A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecol Lett 8:148–159

Chung H, Zak DR, Lilleskov EA (2006) Fungal community composition and metabolism under elevated CO_2 and O_3. Oecologia 147:143–154

Colwell RK (2006) EstimateS: statistical estimation of species richness and shared species from samples. Version 8.0. User's Guide and application. http://purl.oclc.org/estimates. Accessed 1 July 2009

Dickson RE, Lewin KF, Isebrands JG, Coleman MD, Heilman WE, Riemenschneider DE, et al (2000) Forest Atmosphere Carbon Transfer and Storage (FACTS-II) the Aspen Free-air CO2 and O3 Enrichment (FACE) Project: an Overview. Gen. Tech. Rep. NC-214. St. Paul, MN: USDA-FS, NCRS

Dosskey MG, Linderman RG, Boersma L (1990) Carbon–sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. New Phytol 115:269–274

Edwards IP, Zak DR (2011) Fungal community composition and function after long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3. Glob Chang Biol 17:2184–2195

Finzi AC, Norby RJ, Calfapietra C, Gallet-Budynek A, Gielen B, Holmes WE, Hoosbeek MR, Iversen CM, Jackson RB, Kubiske ME et al (2007) Increases in nitrogen uptake rather than nitrogen-use-efficiency support higher rates of temperate forest productivity under elevated CO_2. PNAS 104:14014–14019

Fransson P (2012) Elevated CO_2 impacts ectomycorrhiza-mediated forest soil carbon flow: fungal biomass production, respiration and exudation. Fungal Ecol 5:85–98

Fransson PMA, Taylor AFS, Finlay RD (2005) Mycelial production, spread and root colonization by the ectomycorrhizal fungi *Hebeloma crustuliniforme* and *Paxillus involutus* under elevated atmospheric CO_2. Mycorrhiza 15:25–31

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gardes M, Bruns TD (1996) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Can J Bot 74:1572–1583

Giovannetti G, Roth-Bejerano N, Zanini E, Kagan-Zur V (1994) Truffles and their cultivation. Hortic Rev 16:71–108

Gorissen A, Kuyper TW (2000) Fungal species-specific responses of ectomycorrhizal Scots pine (*Pinus sylvestris*) to elevated [CO_2]. New Phytol 146:163–168

Hobbie EA, Agerer R (2010) Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. Plant Soil 327:71–83

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792

Johansson EM, Fransson PMA, Finlay RD, van Hees PAW (2009) Quantitative analysis of soluble exudates produced by ectomycorrhizal roots as a response to ambient and elevated CO2. Soil Biol Biochem 41:1111–1116

Kauserud H, Heegaard E, Büntgen U, Halvorsen R, Egli S, Senn-Irlet B, Krisai-Greilhuber I, Dämon W, Sparks T, Nordén J, Høiland K, Kirk P, Semenov M, Boddy L, Stenseth NC (2012) Warming-induced shift in European mushroom fruiting phenology. PNAS 109:14488–14493

Kjøller R, Clemmensen KE (2009) Belowground ectomycorrhizal fungal communities respond to liming in three southern Swedish coniferous forest stands. For Ecol Manag 257:2217–2225

Kõljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E et al (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 166: 1063–1068

Kraepelin G, Michaelis H (1997) Effect of liming on the population of macrofungi in a Scots pine plantation of the Grunewald forest of Berlin. Z Mykologie 63:99–126

Kubiske ME, Quinn VS, Marquardt PE, Karnosky DF (2007) Effects of elevated atmospheric CO_2 and/or O_3 on intra- and interspecific competitive ability of aspen. Plant Biol 9:342–355

Landeweert R, Leeflang P, Kuyper TW, Hoffland E, Rosling A, Wernars K, Smit E (2003) Molecular identification of ectomycorrhizal mycelium in soil horizons. Appl Environ Microbiol 69:327–333

Legendre P, Legendre L (2012) Numerical Ecology. Elsevier, Oxford

Lilleskov EA (2005) How do composition, structure, and function of mycorrhizal fungal communities respond to nitrogen deposition and ozone exposure? In: Dighton J, Oudemans P, White J (eds) The fungal community: its organization and role in the ecosystem, 3rd edn. Marcel Dekker, New York, pp 769–801

Lilleskov EA, Parrent JL (2007) Can we develop general predictive models of mycorrhizal fungal community–environment relationships? New Phytol 174:250–256

Lilleskov EA, Fahey TJ, Lovett GM (2001) Ectomycorrhizal fungal aboveground community change over an atmospheric nitrogen deposition gradient. Ecol Appl 11:397–410

Lilleskov EA, Bruns TD, Horton TR, Taylor D, Grogan P (2004) Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. FEMS Microbiol Ecol 49:319–332

Lilleskov EA, Hobbie EA, Horton TR (2011) Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecol 4:174–183

Lindahl BD, de Boer W, Finlay RD (2010) Disruption of root carbon transport into forest humus stimulates fungal opportunists at the expense of mycorrhizal fungi. ISME J 4:872–881

Nara K, Nakaya H, Hogetsu T (2003) Ectomycorrhizal sporocarp succession and production during early primary succession on Mount Fuji. New Phytol 158:193–206

Parrent JL, Vilgalys R (2007) Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO_2 and nitrogen fertilization. New Phytol 176:164–174

Parrent JL, Morris WF, Vilgalys R (2006) CO_2-enrichment and nutrient availability alter ectomycorrhizal fungal communities. Ecology 87: 2278–2287

Peay KG, Bruns TD, Kennedy PG, Bergemann SE, Garbelotto M (2007) A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. Ecol Lett 10:470–480

Pena R, Offermann C, Simon J, Naumann PS, Geβler A, Holst J, Dannenmann M, Mayer H, Kögel-Knabner I, Rennenberg H et al (2010) Girdling affects ectomycorrhizal fungal (EMF) diversity and reveals functional differences in EMF community composition in a beech forest. Appl Environ Microbiol 76:1831–1841

Phillips RP, Meier IC, Bernhardt ES, Grandy AS, Wickings K, Finzi AD (2012) Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. Ecol Lett 15:1042–1049

Poznanovic SK, Lilleskov EA, Webster CR (submitted) Ectomycorrhizal fungal communities of eastern hemlock and yellow birch seedlings co-occurring on coarse woody debris: composition, species sharing, and relation to host and substrate condition. Mycorrhiza

Pregitzer KS, Loya W, Kubiske M, Zak D (2006) Soil respiration in northern forests exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 148:503–516

Rey A, Jarvis PG (1997) Growth response of young birch trees (*Betula pendula* Roth.) after four and a half years of CO_2 exposure. Ann Bot 80:809–816

Rineau F, Garbaye J (2009) Effects of liming on ectomycorrhizal community structure in relation to soil horizons and tree hosts. Fungal Ecol 2:103–109

Rosling A, Landeweert R, Lindahl BD, Larsson K-H, Kuyper TW, Taylor AFS, Finlay RD (2003) Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. New Phytol 159:775–783

Shaw PJA, Dighton J, Poskitt J, McLeod AR (1992) The effects of sulphur dioxide and ozone on the mycorrhizas of Scots pine and Norway spruce in a field fumigation system. Mycol Res 96:785–791

Staddon PL, Fitter AH (1998) Does elevated atmospheric carbon dioxide affect arbuscular mycorrhizas? Trends Ecol Evol 13:455–457

Taylor AFS, Finlay RD (2003) Effects of liming and ash application on below ground ectomycorrhizal community structure in two Norway spruce forests. Water Air Soil Pollut Focus 3:63–76

Tedersoo L, Hansen K, Perry BA, Kjøller R (2006) Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytol 170:581–596

Twieg BD, Durall DM, Simard SW (2007) Ectomycorrhizal fungal succession in mixed temperate forests. New Phytol 176:437–447

Wallenda T, Kottke I (1998) Nitrogen deposition and ectomycorrhizas. New Phytol 139:169–187

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: a guide to methods and applications. Academic, New York, pp 315–322

Yarwood SA, Myrold DD, H gberg MN (2009) Termination of below-ground C allocation by trees alters soil fungal and bacterial communities in a boreal forest. FEMS Microbiol Ecol 70:151–162