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Aspen-associated mycorrhizal fungal production and respiration as a function of changing CO_2, O_3 and climatic variables

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ABSTRACT

The relationships of mycorrhizal fungal respiration and productivity to climate and atmospheric chemistry remain under characterized. We quantified mycorrhizal sporocarp and hyphal respiration, as well as growing season net hyphal production, under ambient and elevated carbon dioxide (CO_2) and ozone (O_3) in relation to natural temperature and moisture variation. Hyphal respiration did not respond significantly to elevated CO_2 and O_3. Sporocarp respiration was affected by temperature and moisture content while hyphal respiratory response to temperature was undetected over the narrower range of soil temperatures captured. Hyphal respiration comprised 31 % of soil respiration, and the ratio of hyphal respiration to soil respiration declined with elevated CO_2. Hyphal biomass was reduced under all treatments though not statistically significant. Given the large fraction of soil respiration represented by mycorrhizal fungi and its sensitivity to climate, a small change in fungal respiration could strongly affect carbon budgets and cycling under climate change.

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Introduction

Understanding the regulators of soil respiration is critical for our ability to model ecosystem carbon (C) cycling within a global change context. Although traditionally not executed, the components of soil respiration should be partitioned into autotrophic and heterotrophic sources, with the latter encompassing organisms directly associated with autotrophs (such as rhizosphere-associated organisms, including mycorrhizal fungi) as well as free-living heterotrophic organisms (such as saprotrophs). Partitioning the heterotrophic and autotrophic components of soil respiration in field studies can be quite challenging (Ekblad et al., 2013; Hanson et al., 2000; Heinemeyer et al., 2011), with the fungal component of soil respiration rarely quantified (but see Heinemeyer et al., 2007; Heinemeyer et al., 2012).

Fungal respiration by different tissue types (e.g., hypha, mycorrhiza and sporocarp) is even less quantified, even though mycorrhizal fungi comprise a significant portion of microbial biomass within forest soils (Cairney, 2012; Ekblad et al., 2013; Högberg and Högberg, 2002; Wallander et al., 2001). Net primary production (NPP) allocated to the fungal components of mycorrhizal fungi ranges from less than 5 % to, more commonly, around 20 % (Hobbie, 2006; Smith and Read, 2008 and references within). Considering that 27–67 % of NPP is partitioned as belowground NPP (BNPP) (Hobbie, 2006), mycorrhizal fungi clearly represent a large fraction of BNPP. Hence, their growth and activities should represent a significant source of CO_2 flux from ecosystems.

Atmospheric change, whether physical or chemical, can affect carbon cycling by altering production, storage, allocation, or respiration (Comstedt et al., 2006; Karnosky, 2003; Karnosky et al., 2005; King et al., 2001; Loya et al., 2003; Miller and Fitzsimmons, 2011; Podila et al., 2011; Pregitzer et al., 2008; Schlesinger and Lichter, 2001). If elevated levels of CO_2 or O_3 influence how primary producers gain and allocate photosynthate to belowground structures, including the supply of carbon to their fungal symbionts, then the end result could be a change in ecosystem C storage. While increased CO_2 typically amplifies NPP, O_3 acts in an opposing manner and will, at least initially, dampen such effects (Karnosky et al., 2003). Studies of enhanced CO_2 and O_3 concentrations within Free-Air Carbon dioxide Enrichment (FACE) systems have already found effects on mycorrhizal fungi, especially at the community level and in the production of sporocarps (Andrew and Lilleskov, 2009; Parrent et al., 2006; Parrent and Vilgalys, 2007; Podila et al., 2011). Consequently, any change in mycelial production and respiration due to altered CO_2 or O_3 concentrations could affect future soil C sequestration (Alberton et al., 2005; Andersen, 2003; Fransson, 2012; Pickles et al., 2012; Rygiewicz and Andersen, 1994; Treseder and Allen, 2000; Schlesinger and Andrews, 2000) as well as the retention of fungal derived C in the soil.

It is important to note that respiration rates are strongly affected by both temperature and moisture (Heinemeyer et al., 2007, 2012; Koch et al., 2007; López-Gutiérrez et al., 2008; Malcolm et al., 2008). While the effect of temperature is broadly captured in Q10 values, reviews of soil respiration literature have indicated that Q10 values are not constant with changing temperature. This limits the conceptual adequacy of a single Q10 for modeling respiration (Davidson et al., 2006; Lloyd and Taylor, 1994). A variety of factors, such as biochemical reaction rates, physiological acclimation, substrate limitation, thermal stress and moisture stress can alter temperature respiration relationships (Davidson et al., 2006). Although much effort has been applied to characterizing temperature–respiration relationships of soils (Boone et al., 1998; Davidson et al., 2006; Kätterer et al., 1998; Lloyd and Taylor, 1994; Winkler et al., 1996), much less has been applied to field studies of fungal temperature–respiration relationships.

Water availability additionally affects soil respiration and can confound estimates of temperature effects (Davidson et al., 2006). Surprisingly, very little is known about moisture impacts on field respiration rates of fungi, although they appear to be physiologically active, albeit at very low rates, at lower water potentials than bacteria (Wilson and Griffin, 1975). It is important to quantify field respiration rates in order to better understand how site variables, such as temperature and moisture, can interact to affect fungal contributions to ecosystem respiration.

The objectives of this study were to: (1) quantify the effects of changes in atmospheric carbon dioxide (CO_2) and ozone (O_3) concentrations on mycorrhizal fungal sporocarp and hyphal respiration *in vivo*; while (2) simultaneously quantifying the effect of natural variation in temperature and water availability on fungal respiration; and to (3) determine treatment effects on net hyphal biomass production. We hypothesized that: (1) fungal respiration would increase under elevated CO_2 and decrease under elevated O_3; (2) fungal respiration would increase under higher temperatures, and decrease as a result of lower water availability; and (3) high CO_2 treatments would increase hyphal biomass production, and elevated O_3 would decrease hyphal biomass production.

Materials and methods

Study area

The Aspen FACE study began in 1997 with the trees planted from seedling stage. It was located on the Harshaw Experimental Farm of the USDA Forest Service, Wisconsin, USA (45° 40′ 48″ N, 89° 37′ 48″ W). The climate is cool continental with summer temperatures averaging 18.3 °C and an average of 106.7 mm of precipitation falling per month from Jul. to Sep.. Prior to the implementation of a forestry research site in the early 1970's, the land was a potato farm. Hybrid poplar and larch trees were grown until development of the FACE study design. Properties of the sandy loam soil graded along a north-south gradient. This was accounted for by incorporating a blocking design (Dickson et al., 2000). Soil carbon content averaged 1.7 % in 2008 (Andrew & Lilleskov, unpublished).

The study had a randomized complete block design with two factors, CO_2 and O_3, at two levels, leading to the following treatment combinations: ambient, elevated CO_2, elevated O_3, and the combination of elevated $CO_2 + O_3$. These four combinations were replicated in three blocks. Carbon dioxide fumigation levels were fixed at ambient (which increased from 360 to

380 ppm over the course of the study) and elevated (200 ppm + ambient). Ozone levels were fixed at ambient (approximately 33–67 ppb) and elevated (1.5× ambient; approximately 50–100 ppb). The fumigation treatments occurred throughout the growing season, from mid-May to mid-Oct..

Hyphal production measurement

Net growing season hyphal production was characterized using hyphal ingrowth mesh bags (Wallander et al., 2001, 2004) with modifications. Cylindrical bags (9 cm depth) were made using 50 μm nylon mesh fabric (Sefar America, Depew, NY) filled with approximately 130 g of dry sand. Dry sieved (250 μm < particle size < 2 mm) pasteurized C-horizon sand from the Aspen FACE site was used to fill the mesh bags. The sand contained 0.7% organic matter based on loss on ignition. Saprotrophic fungal presence was presumably minimized due to low quantity and quality of C in the sand. It is possible that both arbuscular mycorrhizal and ectomycorrhizal fungi colonized these bags; however, hyphae in similar bags at the Aspen FACE site over the same period had very low abundance of arbuscular fungal marker phospholipids 16:1ω5c relative to the markers for ectomycorrhizal fungi (R.M. Miller, unpublished).

Six hyphal mesh sample bags were placed within each aspen section of the treatment plots between May 23 and 26, 2008. Each bag was placed vertically into the ground and covered with 1 cm of soil with litter redistributed on top. Three days prior to respiration measurements, an equal number of control bags ("blank" bags) were placed 1–3 m from a matching hyphal ingrowth bag. The blank bags equilibrated their CO_2 with the surrounding soil, and as such, they provided a background abiotic soil CO_2 flux rate to correct for disequilibrium CO_2 conditions between the bags and Li-Cor chambers during measurements (Fig 1).

Ingrowth hyphal bag respiration measurements

Respiration measurements occurred between Sep. 19 and 23, 2008, approximately 4 months after originally placing the hyphal bags into the field. Soil and air temperatures were recorded for each sample to control for temperature variability among samples. No measurable rainfall occurred during data collection.

Each bag was gently removed from the soil and brushed clean. The respiration of each bag was measured within 2 min of extracting them from the soil, using a LI-8100 Automated Soil CO_2 Flux System (LI-COR, 2005). Hyphal bags were placed in a 10 cm PVC cap and the respiration chamber was placed over the cap. A wire structure held the bags in the cap with two loops at each end, so that the bag was suspended above the bottom of the cap, minimizing resistance to CO_2 efflux from the bag. This was done so the bags would equilibrate with chamber conditions as rapidly as possible. The observation length was 2 min after a 30 s deadband (which helped equilibrate chamber conditions). Hyphal bag temperature was recorded prior to the respiration measurement.

Hyphal respiration per bag was calculated by subtracting the blank bag CO_2 flux value from the paired hyphal bag value. Blank respiration fluxes were always less than ingrowth bags, and were on average 44% of their paired bags. To minimize

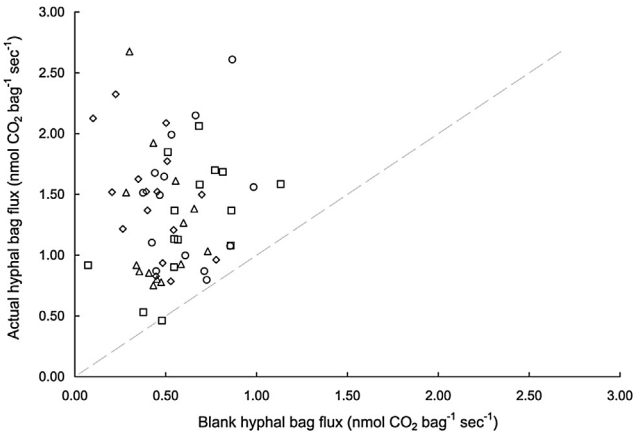

Fig 1 – Carbon dioxide flux of blank versus hyphal bags exposed to ambient or elevated CO_2 and/or O_3. The blank bags correct for disequilibrium conditions during respiration measurements. The dotted line represents a 1:1 ratio of blank bag flux to hyphal bag flux, as would occur if there were no respiratory CO_2 flux. Values above that indicate greater CO_2 flux from the hyphal bags than the blank bags. Triangles are ambient treatments, circles are elevated CO_2, diamonds are elevated O_3 and squares are elevated CO_2 + O_3.

the effect of disequilibrium conditions created by removing the bags from the soil, we used the Type 2 fit of CO_2 concentration versus temperature calculated by the LI-8100:

$$dC'/dt = a\left(C'_X - C'_0\right)e^{-a(t-t_0)}$$

where C' is the CO_2 concentration, a is a parameter that defines the curvature, C'_x is a parameter that defines the asymptote, C'_0 is the value of C′ when the chamber is closed, e is the base of the natural logarithm, t is the time at which the rate is to be calculated, and t_0 is the time of chamber closure (LI-COR, 2005). Parameters a, C'_x and C'_0 are all fitted by the LI-8100 for each flux measurement. By substituting the time at the end of the sample period (t = 150 s) in this equation, we estimated the instantaneous flux rate at that time, instead of the default rate reported by the LI-8100, which is estimated at the time of chamber closure (t_0) when flux disequilibrium is greatest. Blank-corrected flux rates of the sample bags calculated at 150 s were on average 85% of initial (t_0) rates.

Following respiration measurements, the hyphae were extracted by pouring the bag contents into 700–1 000 ml of distilled water, swirling, and decanting over a wire sieve lined with 50 μm nylon mesh. The process was then repeated. The hyphae, as well as a minor amount of adhering sand that would not easily separate from the hyphae, were stored at −80 °C until further processing.

The collected hyphae were freeze dried, weighed and ground into a fine powder using a bead beater and glass beads (3 mm diameter; 2 min at high speed). ^{13}C and % C were obtained using a ThermoFinnigan Delta^{plus} Continuous-Flow Stable Isotope Ratio Mass Spectrometer with a GasBench II and a Costech 4010 Elemental Analyzer at the Ecosystem Science Center, Michigan Technological University. Hyphal weights were corrected for sand content using the % C of

hyphae, assumed to be 39 % C based on C analyses of sporocarps at the FACE site (A. Piket and E.A. Lilleskov, unpublished). These C values are comparable to values found for fungal sporocarps in other studies (e.g., Hart et al., 2006) as well as arbuscular mycorrhizal fungal (AMF) hyphae (van Diepen et al., 2010).

Isotope analysis of the hyphae within the bags corroborated the assumption that mycorrhizal fungi were the dominant hyphal producers. The $\delta^{13}C$ signature of the hyphae was found to be within the expected range for ectomycorrhizal fungi (Hobbie et al., 2001; Högberg et al., 1999; Wallander et al., 2004). The mean hyphal $\delta^{13}C$ within ambient and elevated O_3 treatments was −25.9 (±0.17 SE) ‰ and −26.3 (±0.05) ‰, respectively. For ectomycorrhizal sporocarps from this study, control plot $\delta^{13}C$ values averaged −25.4, −25.7 and −25.4‰ for *Inocybe lacera*, *Laccaria laccata* and *Leccinum insigne*, respectively. In contrast, *Chalciporus piperatus*, a putatively saprotrophic species, was −22.3‰. After correcting for isotopic signature of the residual sand, the mean hyphal $\delta^{13}C$ within both the elevated CO_2 and elevated $CO_2 + O_3$ treatments was between −32 and −34‰. This was very similar to the average isotopic signature of ectomycorrhizal sporocarps for those treatments, which had average values of −35.1‰ and −35.7‰, respectively. The lower isotopic $\delta^{13}C$ signature with elevated CO_2 was due to the use of fossil fuel derived gas for the CO_2 enrichment treatment. We interpret these values as indicating that mycorrhizal fungi were the dominant hyphal producers within the bags. Natural abundance $\delta^{15}N$ could not be used to ascertain fungal ecology as it remained universally elevated due to the addition of isotopically labeled N to the plots in 2003 (Zak et al., 2007).

To obtain estimates of CO_2 flux per hyphal bag, the blank-corrected default Li-8100 per m^2 flux estimates were corrected by rescaling to flux per actual chamber area. The per bag flux estimates were divided by the hyphal weight to obtain a per unit biomass flux rate in nmol mg^{-1} s^{-1}.

Sporocarp respiration

To control for possible species effects, respiration measurements were preferentially taken on taxa present within all four treatment plots within at least one block at the same time, which limited measurements primarily to *L.* c.f. *insigne* (but see Supplementary Appendix 1) and to the 2007 sampling season, as sporocarp production was extremely sparse in 2008. Respiration measurements were randomized by treatment, and by block when taxa were present in more than one block. Within each taxon, sporocarps were selected to keep their condition (e.g., developmental status, damage by fungivores) as similar as possible within a block, and the conditions were recorded (Supplementary Appendix 1). Unlike the hyphal measurements, which were limited to one respiration measurement at the end of the growing season, sporocarp respiration occurred throughout the growing season (Jul. 17th to Sep. 21st 2007).

Sporocarps were harvested intact and cleaned of adhering soil and debris. Temperature and fresh weight were recorded at time of measurement, with water content and dry weight determined later. Respiration measurements were determined within 2 min of harvest by placing a sporocarp within a closed chamber and measuring respiration for 1.5 min after a 30 s deadband, as recommended (LI-COR, 2005). Respiration chamber headspace volume was corrected for sporocarp volume. Depending on sporocarp size, appropriately sized (10 cm or 20 cm diameter) respiration chambers were placed over either a 10 cm closed PVC cap or an 20 cm PVC soil collar with a square of Plexiglas sealed to the bottom of the collar with RTV 3145 Silicone Adhesive (Dow Corning, Midland, MI).

The effect of harvest on sporocarp respiration was investigated by measuring sporocarp respiration values produced by sporocarps growing within a long term soil collar established at the site. Respiration rates were also measured for both the soil and the harvested sporocarps. The respiration values of the harvested sporocarp plus the soil alone equaled the values taken of intact sporocarps within the soil horizon, indicating that harvesting did not affect sporocarp respiration rates within the short time period between harvest and measurement (Andrew & Lilleskov, unpublished).

Soil-mycorrhizal respiratory contribution

To view the effect of treatments on the proportion of soil respiration represented by mycorrhizal hyphae, hyphal respiration (per unit area) was compared to soil respiration measurements (A.J. Burton, personal communication) from within the same time period as our hyphal respiration measurements. The methods for soil respiration measurements have been described in Pregitzer et al. (2008). To compare soil respiration to mycorrhizal respiration we assumed that the mycorrhizal fungal hyphal biomass and respiration in the ingrowth bags was representative of the mycorrhizal fungal hyphal biomass and respiration in the bulk soil. We used the cross sectional area of the ingrowth bags to estimate flux per unit area down to 9 cm in the soil (the length of the bags). Statistical analyses followed the procedures described below.

Statistical analysis

Data analysis was performed partly in R version 2.14.0 (R Development Core Team 2011; Venables and Ripley, 2002), modeling response against the fixed factors CO_2, O_3, block and the interaction of CO_2 and O_3. To account for variation in respiratory CO_2 flux due to temperature and moisture levels, sporocarp CO_2 flux was linearly regressed against CO_2 level, O_3 level, sporocarp temperature, and sporocarp moisture content after log-transforming CO_2 flux. Data analysis on sporocarp respiration was restricted to *L. insigne*, as the other taxa did not fruit in high enough abundance to be adequately analyzed within the FACE treatment design (Supplementary Appendix 1).

To examine an apparent decline in respiration with increasing temperature, respiration of sporocarps of all species and of *L. insigne* above a range of moisture thresholds (80, 85 and 88 %) were fit using unimodal three parameter Gaussian distributions in Sigmaplot version 9.01 (Systat Software, Inc.). Two parameter exponential fits were also carried out with all data and with data from sporocarps at temperatures below 18 °C to explore deviations from exponential relationships with increasing temperatures.

Results

Hyphal production

Net hyphal biomass production over the growing season averaged 9.33 (SE ± 0.46) g m^{-2}. Treatment and block effects on mean production were not significant (CO_2 $p = 0.146$; O_3 $p = 0.235$; CO_2xO_3 $p = 0.805$; block $p = 0.712$). Mean (±SE) hyphal production was 10.79 (±1.73), 9.29 (±0.44), 9.66 (±0.73) and 7.60 (±0.31) g m^{-2} within control, CO_2, O_3, and $CO_2 + O_3$ plots, respectively. Production was 86 %, 90 %, and 70 % of the control plots under elevated CO_2, O_3 and $CO_2 + O_3$, respectively.

Hyphal respiration per unit area

CO_2 and O_3 concentrations did not drive statistically significant changes in hyphal respiration rates on a per unit area basis (CO_2 $p = 0.576$; O_3 $p = 0.931$; CO_2xO_3 $p = 0.298$; block $p = 0.800$). Mean rates were 117 %, 137 % and 85 % of the control under elevated CO_2, elevated O_3 and elevated $CO_2 + O_3$, respectively. Respiration rates to a depth of 10 cm (μmol CO_2 m^{-2} sec^{-1}) averaged 0.91 with a median of 0.75 and standard deviation of 0.60 ($N = 59$). Hyphal respiration scaled up from the ingrowth bags was, on average, 31 % of soil respiration, with values of 35 %, 30 %, 39 %, and 18 % of soil respiration for control, elevated CO_2, elevated O_3, and elevated $CO_2 + O_3$, respectively.

Hyphal and sporocarp respiration on a mass-specific basis

Mass-specific hyphal respiration (nmol mg^{-1} sec^{-1}) averaged 0.144 with a median of 0.141 and standard deviation of 0.085 ($N = 55$). It also was not significantly affected by CO_2 or O_3 levels (CO_2 $p = 0.858$; O_3 $p = 0.532$; $CO_2 \times O_3$ $p = 0.438$; block $p = 0.993$). Hyphal respiration rates under all of the elevated CO_2 and/or O_3 conditions trended to be higher than in the ambient conditions (Fig 2). There was no significant effect of the 4.1 °C range of measurement temperatures on hyphal respiration rate.

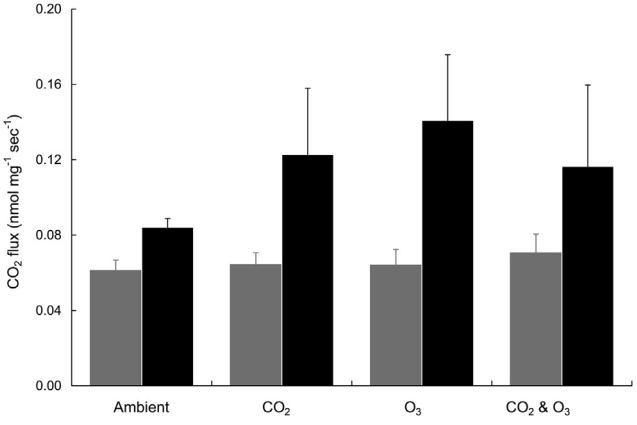

Fig 2 – Mean respiration (with standard error bars) of mycorrhizal sporocarps (gray) and hyphae (black) exposed to ambient or elevated CO_2 and/or O_3. Respiration is expressed on a dry mass basis (nmol CO_2 mg^{-1} sec^{-1}).

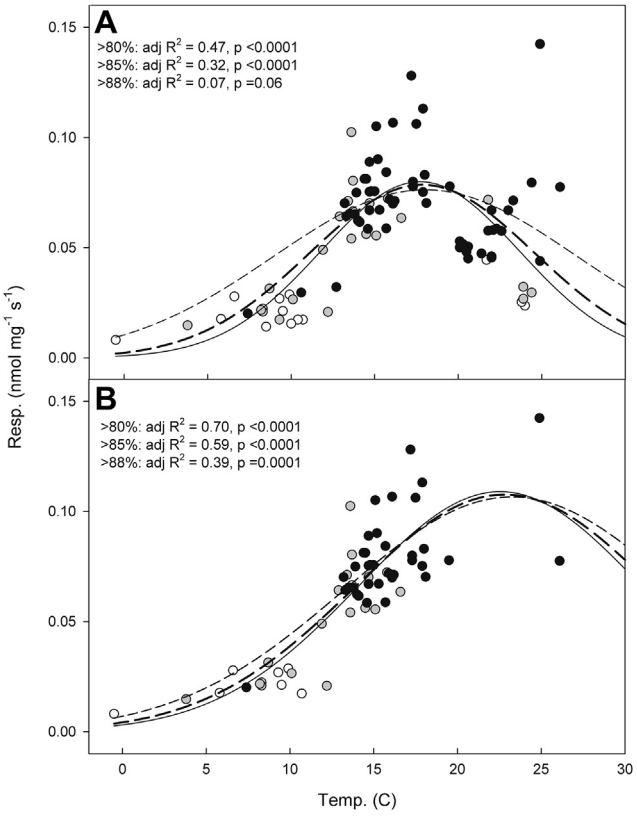

Fig 3 – The relationship of sporocarp temperature and mass-specific respiration for all species (A) and *Leccinum insigne* alone (B). Symbols for individual sporocarps are coded for water content as follows: white = between 80 and 85 %, gray = between 85 and 88 %, black = > 88 %. Lines indicate Gaussian three parameter fits for > 80 % moisture content (solid line), > 85 % moisture content (long dash) and > 88 % moisture content (short dash).

In the regression model that included CO_2, O_3, temperature and moisture, sporocarp mass-specific respiration rates were strongly affected by temperature and moisture content, but were not significantly affected by CO_2 or O_3 concentration (Fig 2; CO_2 $p = 0.476$; O_3 $p = 0.509$; $CO_2 \times O_3$ $p = 0.801$; block $p = 0.155$). A model including sporocarp temperature and percent moisture content explained much of the variation in respiration rates ($R^2_{adj} = 0.856$; Supplementary Appendix 2). Sporocarp respiration was similar across all treatments (Fig 2). Sporocarp respiration rates were 66 % of the hyphal rates, with an overall average rate of 0.061 (±0.004 SE) nmol CO_2 mg^{-1} sec^{-1} and median of 0.067 ($N = 56$). Mean rates were 105 %, 105 % and 115 % of the control under elevated CO_2, elevated O_3 and elevated $CO_2 + O_3$, respectively.

When hyphal and sporocarp respiration rates were compared within the same temperature range (15.8–19.5 °C), *L. insigne* sporocarps respired an average of 0.075 nmol mg^{-1} sec^{-1}, compared to the 0.144 nmol mg^{-1} sec^{-1} reported previously for hyphae. Across all sporocarps under similar temperatures, sporocarp respiration rates by plot were, on average, 75 % of the hyphal rates (±8 % SE). Treatment comparisons showed rates at 101 %, 95 % and 111 % of the control under elevated CO_2, elevated O_3 and elevated $CO_2 + O_3$, respectively.

Respiration of all sporocarps combined was strongly influenced by both temperature and moisture. When compared across a range of sporocarp moisture thresholds (>80 %, >85 %, and >88 %), temperature–respiration relationships were best described by a unimodal Gaussian regression in which respiration increased rapidly at lower temperatures, and then declined above approximately 18 °C (Fig 3A). Taxon-specific temperature–respiration relationships for *L. insigne* likewise flattened out at higher temperatures (Fig 3B), although there were fewer data points at this range. Simple exponential models that would approximate a standard Q10 relationship fit the data poorly when higher temperature data were included (large negative residuals at low temperatures, and $R^2 = 0.11$ for all sporocarps, 0.54 for *L. insigne*) whereas when only temperatures below 18 °C were included, model fits were much better ($R^2 = 0.72$ for all sporocarps, 0.70 for *L. insigne*; Fig 4).

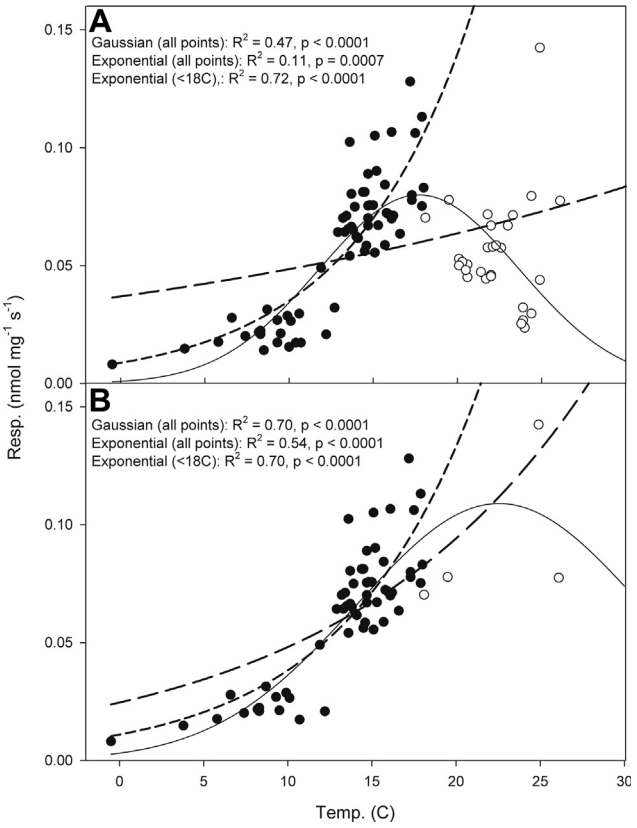

Fig 4 – The relationship of sporocarp temperature and mass-specific respiration for sporocarps >80 % moisture content, for all species (A) and *Leccinum insigne* (B). Black symbols are <18 °C, white symbols are >18 °C. The data are fitted with three parameter Gaussian models for all data points (solid line), two parameter exponential models for all data points (long dash), two parameter exponential models for sporocarps <18 °C (short dash).

Discussion

Mycorrhizal respiration and production with elevated CO_2 and O_3

The lack of a hyphal production response to the CO_2 and O_3 treatment contrasts with our earlier finding of significantly increased sporocarp production under elevated CO_2 (Andrew and Lilleskov, 2009). Whether this is due to better sampling of sporocarp than hyphal production, interannual variation, or real differences between sporocarps and hyphae in production responses to the treatments is not certain. We can say that interannual variation in sporocarp production effects can be quite large (Andrew and Lilleskov, 2009; Fig 2). We also acknowledge that the study design of the FACE site, with three replicates per treatment, provided low statistical power to detect a field-based response, so our failure to find a treatment effect should be interpreted with caution. Clearly sporocarp sampling had a larger footprint, with all sporocarps in the plots sampled at biweekly intervals throughout the growing season.

It is also clear that the lack of significant changes in hyphal biomass production under elevated CO_2 is consistent with the handful of previous field studies available (Parrent and Vilgalys, 2007; Godbold et al., 2006; Hagedorn et al., 2013). Parrent and Vilgalys (2007) also found no statistically significant effect of CO_2 enrichment on ectomycorrhizal hyphal biomass within a FACE experiment of even-aged forests of *Pinus taeda*. Our findings are also consistent with the lack of a CO_2 effect on mycorrhizal hyphal inputs into soil C, suggested at FACE experiments with *Populus* species (Godbold et al., 2006) and a mix of *Larix decidua*, *Pinus cembra* and *Pinus mugo* (Hagedorn et al., 2013). These findings indicate that mycorrhizal mycelial production in the field may be less sensitive to the effects of elevated CO_2 and O_3 than in more controlled systems, although this undoubtedly is taxon dependant (Bryla and Eissenstat, 2005; Kasurinen et al., 2005; Parrent and Vilgalys, 2007; Treseder and Allen, 2000; Trocha et al., 2010; Wilkinson et al., 2012). The apparent lack of a significant treatment effect may be a result of greater environmental variability in the field. To our knowledge, this is the first study to document the effects of elevated O_3 on hyphal production in the field.

Mycelial attributes other than production and respiration may be more strongly affected by increased CO_2 or O_3. For example, CO_2 can affect mycelial metabolic activity (Chung et al., 2006; Parrent and Vilgalys, 2009), C allocation to chitin and/or storage compounds (Staddon, 2005), hyphal turnover (Kasurinen et al., 1999), hyphal exudation (Fransson, 2012), or possibly, transformations into other pools due to browsing and consumption (Supplementary Appendix 1). Godbold et al. (2006) hypothesized that, while elevated CO_2 has been found to either positively or to not affect mycorrhizal hyphae (Fransson, 2012; Treseder and Allen, 2000), when there is a lack of biomass change it is usually accompanied by a lack of compositional change. This could indicate some taxa are more resistant to changing CO_2 concentrations than others.

Comparison of sporocarp and hyphal tissue respiration

It is interesting to note that sporocarp respiration rates were lower than hyphal rates, at 75 % of the latter when analyzed within the same temperature range. It is possible that our method for hyphal respiration slightly overestimates rates if

we did not fully correct for disequilibrium between the bags and chamber, but the long thin ingrowth bags were designed to minimize resistance to efflux, leading to more rapid equilibration with the flux chamber; our use of the rates from the end of the flux measurement period gave more time for equilibration; and the blank correction should have corrected for residual disequilibrium conditions. Assuming the rates are correct, perhaps metabolic activity is lower in sporocarps than hyphae, as the former are composed largely of structural tissue designed to support spore production while the latter are responsible for exploratory growth, metabolically costly nutrient extraction and conversion to organic forms (Wallander, 1995), and even mushroom primordia formation. Reproductive structures may initially be costly to create due to the high amounts of proteins and lipids (Bryla and Eissenstat, 2005), but once the energy has been invested they may respire considerably less than soil mycelium.

It is also possible that sporocarp respiration is reduced because sporocarps undergo more intense desiccation as a result of exposure to high vapor pressure deficits in the ambient atmosphere compared with soil, resulting in higher evaporative water loss (Lilleskov et al., 2009). In the same way that water potential across the soil-plant-atmosphere continuum (SPAC; Philip, 1966) is lowest in the leaves, the subaerial parts of fungi (i.e., sporocarps sampled in the present study) should, under most conditions, have lower water potential than the hyphae that supply them. By analogy, we could refer to fungal water relations in this context as occurring in the soil-fungus-atmosphere-continuum (SFAC), although for mycorrhizal species the direct water interactions with the plant via mycorrhizal linkages would also have to be taken into consideration.

Although the data are noisy, it appears that at higher temperatures the sporocarp temperature response diminishes greatly (Fig 3). This may not be due solely to moisture limitation because it occurs in moisture-sufficient sporocarps. We cannot say whether this is due to inherent physiology of the fungi or to limitations driven by resources (e.g., carbon). Most fungi have a unimodal response to temperature, with growth and respiration increasing nearly exponentially at lower temperatures, then peaking and declining as temperature stress on physiology becomes too great (e.g., Dejardin and Ward, 1971). The shape of this curve is strain-specific, defined by thermal adaptations to low and high temperatures (e.g., Prasad et al., 1979). In addition to these inherent thermal limits, fungal respiration might also be limited by carbon supply. Mycorrhizal fungi are dependent on hosts for most of their carbon. As host respiration increases with temperature we would expect supplies of carbon available for transfer to symbionts to decline at the same time that fungal demand is at its greatest (Pregitzer et al., 2000; Atkin et al., 2000). Evidence does exist for a greater linkage of mycorrhizal fungal respiration with photosynthate substrate supply than with temperature (Heinemeyer et al., 2007). Further experiments or modeling would be required to determine which of these likely explains the flat temperature response at higher temperatures.

The response of sporocarp and hyphal respiration to temperature was not similar, partly because the temperature range of measurement was much larger for sporocarps (−0.5 °C–26.1 °C) than for hyphae (14.1 °C–18.6 °C). As such, the lack of a strong temperature control over hyphal respiration may be attributable to the small temperature range of the soil during measurements, which only varied by 4.1 °C. Heinemeyer et al. (2007) similarly found little evidence of soil temperature affecting hyphal respiration across a much greater temperature range, probably due to regulation by photosynthate supply. Hyphal respiratory responses to temperature may also acclimate to changing environments (Malcolm et al., 2008, 2009; López-Gutiérrez et al., 2008). If the response of mycorrhizal respiration to temperature is consistently muted, this has important implications for climate models.

Contribution of hyphal respiration to soil respiration

We found that hyphal respiration (to a depth of 9 cm) ranged from 18 % to 39 % of soil respiration, with an overall mean of 31 %. Although, to our knowledge, root respiration has not been quantified at the Aspen FACE site, literature estimates for belowground autotrophic respiration ($R_{a\text{-}soil}$) are available. $R_{a\text{-}soil}$, which is really a combination of root, mycorrhizal and exudate-derived respiration, range from 47 % to 60 % of soil respiration. Högberg et al., 2001 using a girdling approach estimated $R_{a\text{-}soil}$ at about 54 % of soil respiration. Bond-Lamberty et al. (2004) and Hanson et al. (2001) both also estimated $R_{a\text{-}soil}$ at approximately 50 % of soil respiration. Similarly, Griffis et al. (2004) reported root respiration as 47 % of soil respiration in a mixed aspen stand, but this was derived from the equations of Bond-Lamberty et al. (2004). Yang and Chuankuan (2006) also estimated autotrophic respiration at 47 % of soil respiration, while Heinemeyer et al. (2012) obtained an estimate of 56 %. Using the difference between soil respiration and aboveground net primary production, Russell and Voroney (1998) estimated $R_{a\text{-}soil}$ (the "root fraction") at 60 % of soil respiration in a boreal *Populus tremuloides* stand. Subtracting the average percent of hyphal respiration at the Aspen FACE site (31 %) from the maximum (60 %) and minimum (47 %) values of $R_{a\text{-}soil}$ leaves 16–29 % of soil respiration that can be attributed to root respiration, mycorrhizal respiration deeper in the soil, and respiration of exudates. Estimates of aspen mass-specific root respiration from other studies (e.g., DesRochers et al., 2002) range from 0.003 to 0.008 nmol mg^{-1} sec^{-1}, which is about an order of magnitude lower than mean fungal mass-specific respiration rates in the present study (Table 1). Thus, even small changes in fungal biomass in these systems could lead to large shifts in the mycorrhizal component of soil respiration.

Comparison with respiration rates in other studies

The respiration estimates documented here for both hyphae and sporocarps fit well within the range of values that have been obtained by other, mostly *in vitro*, studies (Table 1). Other researchers have calculated fungal respiration within a range from $2.9e^{-7}$ to 0.22 nmol mg^{-1} sec^{-1}. The hyphal respiration values documented here (~0–0.56 nmol mg^{-1} sec^{-1} for sporocarps and 0.008–0.14 nmol mg^{-1} sec^{-1} for hyphae) increase the threshold of estimated values, but perhaps this should be unsurprising as the values were determined in a

Table 1 – Comparison of biomass-specific respiration rates of fungi[a]

Study reference	Fungal organism	Type of structure	Type of experiment	Ecology	CO_2 flux (nmol CO_2 mg^{-1} sec^{-1})
Heinemeyer et al., 2006	*Glomus mosseae*	Hyphae	Hyphal compartment	Arbuscular mycorrhizal	$2.9e^{-7}$ to $1.9e^{-5}$
Varoquaux et al., 1999	*Agaricus bisporus*	Sporocarp	Cultivated	Saprotrophic	0.0003 to 0.0006
Rivera et al., 2010	*Tuber* species	Sporocarp	Field; delayed measurement	Ectomycorrhizal	0.0006 to 0.0027
Koch et al., 2007	*Piloderma croceum*	Hyphae	Culture	Ectomycorrhizal	0.0018 to 0.0221
Ares et al., 2006	*Lentinula edodes*	Sporocarp	Cultivated	Saprotrophic	0.004
Ettema et al., 1999	Unknown fungal taxa	Hyphae	Incubation	Unknown (probably saprotrophic)	0.005 to 0.007
Malcolm et al., 2008; 2009	11 ectomycorrhizal species[b]	Hyphae	Culture	Ectomycorrhizal	0.010 to 0.150
Rygiewicz and Andersen, 1994	*Hebeloma crustuliniforme*	Hyphae	Microcosm	Ectomycorrhizal	0.011 to 0.016
Boström et al. 2008	7 ectomycorrhizal species[c]	Sporocarp	Field	Ectomycorrhizal	0.017 to 0.222
Malcolm et al., 2008; 2009	Modeled estimate	n/a	Model (equation)	n/a	0.024
Ek, 1997	*Paxillus involutus*	Hyphae	Microcosm	Ectomycorrhizal	0.027 to 0.076
Hammond and Nichols, 1975	*Agaricus bisporus*	Sporocarp	Cultivated	Saprotrophic	0.028 to 0.063
Boström et al. 2008	9 Saprophytic species[d]	Sporocarp	Field	Saprotrophic	0.034 to 0.139
Boufalis and Pellissier, 1994	*Laccaria laccata* & *Cenococcum graniforme*	Hyphae	Culture	Ectomycorrhizal	0.035 to 0.065
Souto et al., 2000	*Hymenoscyphus ericae* & *Hebeloma crustuliniforme*	Hyphae	Culture	Ectomycorrhizal	0.036 to 0.086
Eltrop and Marschner, 1996	*Pisolithus tinctorius*	Hyphae	Microcosm	Ectomycorrhizal	0.085 to 0.223
Braga et al., 1999	*Metarhizium anisopliae*	Hyphae	Culture	Parasitic entomopathogen	0.11
This study	Unknown mycorrhizal taxa	Hyphae	Field	Ectomycorrhizal	−0.0015 to 0.5565
This study	*Leccinum* c.f. *insigne*	Sporocarp	Field	Ectomycorrhizal	0.008 to 0.142

a Adapted from van Diepen (2008). The biomass-specific measures reported here prohibit area-based measures from being reported.
b *Amanita muscaria* var. *muscaria*, *A. muscaria* var. *formosa*, *A. citrina*, *Cenococcum geophilum*, *Lactarius* sp., *L.* cf. *pubescens*, *L. chrysorrheus*, *Leccinum* cf. *alaskanum*, *Lec. aurantiacum*, *Suillus intermedius*, *S.* cf. *grevillei*.
c *Gomphidius glutinosus*, *Cantharellus cibarius*, *Paxillus involutus*, *Amanita muscaria*, *Boletus subtomentosus*, *Elaphomyces muricatus*, *Hygrophorus piceae*.
d *Micromphale perforans*, *Lycoperdon perlatum*, *Pholiota alnicola*, *Hypholoma capnoides*, *Piptoporus betulinus*, *Fomes fomentarius*, *Fomitopsis pinicola*, *Ganoderma applanatum*, *Trametes hirsuta*.

field setting where greater variability occurs. Additionally, the range of 0.008–0.14 nmol CO_2 mg^{-1} sec^{-1} respiration for ectomycorrhizal sporocarps is quite similar to the sporocarp respiration values Boström et al. (2008) measured in seven ectomycorrhizal fungal species within the field (0.017–0.222 nmol CO_2 mg^{-1} sec^{-1}).

Heinemeyer et al. (2007, 2012) provide what are probably the most appropriate values of *in situ* hyphal respiration with which to compare the data presented here. The estimates of mycorrhizal respiration from Heinemeyer et al. (2007) were substantially less than ours, ranging from 0.28 to 0.44 μmol CO_2 m^{-2} sec^{-1} during three trial periods, while our estimates averaged 0.91 μmol CO_2 m^{-2} sec^{-1} (with a range of −0.02–2.72 μmol CO_2 m^{-2} sec^{-1}). However, as a percent of soil respiration, contributions of mycorrhizal hyphal respiration were quite similar. Heinemeyer et al. (2007) calculated an approximately 25% seasonal contribution of ectomycorrhizal fungi to soil respiration during their first trial, very similar to the 31% we report here. A contribution between 8% and 22% over the growing season was calculated in another study (Heinemeyer et al., 2012). Lastly, 8–22% contribution was calculated by Hasselquist et al. (2012), in which the highest percentage was found within low N plots, this being the most similar to our 31% value. Potential methodological differences between these studies and our own include: our measurements were taken late in the growing season after aboveground sinks have been satisfied and belowground allocation is typically higher. Additionally, host species, soil moisture, soil type and temperatures all differed among the studies. Regardless of these differences, these studies point to relative uniformity of the proportion of hyphal respiration to total soil respiration.

Conclusions

Whereas studies of sporocarp production indicated that the transfer of carbon to the fungus is increased under elevated CO_2 and, at least initially, decreased under elevated O_3 (Andrew and Lilleskov, 2009), this pattern does not hold true for mycelial production within the same study system. This suggests that sporocarp production may be more sensitive to changing atmospheric chemistry than is hyphal production.

There is potential for C cycling within forest ecosystems to be affected by relatively small changes in biomass-specific respiration rates of ectomycorrhizal fungi in response to changes in climate and atmospheric chemistry. Combining the results here with those found within other natural systems, especially other climate change experiments, will further

clarify the determinants of mycorrhizal respiration, hyphal productivity and their contribution to ecosystem C pools. It is important to note that there is a strong effect of temperature and moisture on ectomycorrhizal fungal productivity and respiration, regardless of CO_2 and O_3 level. Furthermore, temperature effects appear to plateau or even possibly decline at field-relevant temperatures. In addition, fungal respiration appears to be much higher than root respiration on a mass-specific basis. As a result, future greenhouse gas mediated climate change could strongly affect mycorrhizal fungal contributions to C cycling. Better quantification of these contributions will aid our ability to predict and model impacts of global change on terrestrial C cycling.

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Supplementary data

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REFERENCES

Alberton, O., Kuyper, T.W., Gorissen, A., 2005. Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO_2. *New Phytologist* 167, 859–868.

Andersen, C.P., 2003. Source-sink balance and carbon allocation below ground in plants exposed to ozone. *New Phytologist* 157, 213–228.

Andrew, C., Lilleskov, E.A., 2009. Productivity and community structure of ectomycorrhizal fungal sporocarps under increased atmospheric CO_2 and O_3. *Ecology Letters* 12, 813–822.

Ares, G., Parentelli, C., Gámbaro, A., Lareo, C., Lem, P., 2006. Sensory shelf life of shiitake mushrooms stored under passive modified atmosphere. *Postharvest Biology and Technology* 41, 191–197.

Atkin, O.K., Edwards, E.J., Loveys, B.R., 2000. Response of root respiration to changes in temperature and its relevance to global warming. *New Phytologist* 147, 141–154.

Bond-Lamberty, B., Wang, C., Gower, S.T., 2004. A global relationship between the heterotrophic and autotrophic components of soil respiration? *Global Change Biology* 10, 1756–1766.

Boone, R.D., Nadelhoffer, K.J., Canary, J.D., Kaye, J.P., 1998. Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature* 396, 570–572.

Boström, B., Comstedt, D., Ekblad, A., 2008. Can isotopic fractionation during respiration explain the ^{13}C-enriched sporocarps of ectomycorrhizal and saprotrophic fungi? *New Phytologist* 177, 1012–1019.

Boufalis, A., Pellissier, F., 1994. Allelopathic effects of phenolic mixtures on respiration of two spruce mycorrhizal fungi. *Journal of Chemical Ecology* 20, 2283–2289.

Braga, G.U.L., Destéfano, R.H.R., Messias, C.L., 1999. Oxygen consumption by *Metarhizium anisopliae* during germination and growth on different carbon sources. *Journal of Invertebrate Pathology* 74, 112–119.

Bryla, D.R., Eissenstat, D., 2005. Respiratory costs of mycorrhizal associations. In: Lambers, H., Ribas-Carbo, M. (Eds.), *Plant Respiration: From Cell to Ecosystem. Advances in Photosynthesis and Respiration*. Springer, Dordrecht, NL, pp. 207–224.

Cairney, J.W.G., 2012. Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. *Soil Biology & Biochemistry* 47, 198–208.

Chung, H., Zak, D.R., Lilleskov, E.A., 2006. Fungal community composition and metabolism under elevated CO_2 and O_3. *Oecologia* 147, 143–154.

Comstedt, D., Boström, B., Marshall, J.D., Slaney, A.M., Linder, S., Ekblad, A., 2006. Effects of elevated atmospheric carbon dioxide and temperature on soil respiration in a boreal forest using $\delta^{13}C$ as a labeling tool. *Ecosystems* 9, 1266–1277.

Davidson, E.A., Janssens, I.A., Luo, Y., 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q_{10}. *Global Change Biology* 12, 154–164.

Dejardin, R.A., Ward, E.W.B., 1971. Growth and respiration of psychrophilic species of the genus *Typhula*. *Canadian Journal of Botany* 49, 339–347.

DesRochers, A., Landhäusser, S.M., Lieffers, V.J., 2002. Coarse and fine root respiration in aspen (*Populus tremuloides*). *Tree Physiology* 22, 725–732.

Dickson, R.E., Lewin, K.F., Isebrands, J.G., Coleman, M.D., Heilman, W.E., Riemenschneider, D.E., Sober, J., Host, G.E., Zak, D.R., Hendrey, G.R., Pregitzer, K.S., Karnosky, D.F., 2000. Forest Atmosphere Carbon Transfer and Storage (FACTS-II) the Aspen Free-air CO_2 and O_3 Enrichment (FACE) Project: an Overview. USDA-FS, NCRS, St. Paul, MN. General Technical Report NC-214.

Ek, H., 1997. The influence of nitrogen fertilization on the carbon economy of *Paxillus involutus* in ectomycorrhizal association with *Betula pendula*. *New Phytologist* 135, 133–142.

Ekblad, A., Wallander, H., Godbold, D.L., Cruz, C., Johnson, C., Baldrian, P., Björk, R.G., Epron, D., Kieliszewska-Rokicka, B., Kjøller, R., Kraigher, H., Matzner, E., Neumann, J., Plassard, C., 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. *Plant Soil* 366, 1–27.

Eltrop, L., Marschner, H., 1996. Growth and mineral nutrition of non-mycorrhizal and mycorrhizal Norway spruce (*Picea abies*) seedlings grown in semi-hydroponic sand culture. *New Phytologist* 133, 479–486.

Ettema, C.H., Lowrance, R., Coleman, D.C., 1999. Riparian soil response to surface nitrogen input: temporal changes in denitrification, labile and microbial C and N pools, and bacterial and fungal respiration. *Soil Biology and Biochemistry* 31, 1609–1624.

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

Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I.A., Ceulemans, R., Polle, A., Velthorst, E.J., Scarascia-Mugnozza, G., De Angelis, P., Miglietta, F., Peressotti, A., 2006. Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant and Soil* 281, 15–24.

Griffis, T.J., Black, T.A., Gaumont-Guay, D., Drewitt, G.B., Nesic, Z., Barr, A.G., Morgenstern, K., Kljun, N., 2004. Seasonal variation and partitioning of ecosystem respiration in a southern boreal aspen forest. *Agricultural and Forest Meteorology* 125, 207–223.

Hagedorn, F., Hiltbrunner, D., Streit, K., Ekblad, A., Lindahl, B., Miltner, A., Frey, B., Handa, I.T., Hättenschwiler, S., 2013. Nine years of CO_2 enrichment at the alpine treeline stimulates soil respiration but does not alter soil microbial communities. *Soil Biology and Biochemistry* 57, 390–400.

Hammond, J.B.W., Nichols, R., 1975. Changes in respiration and soluble carbohydrates during the post-harvest storage of mushrooms (*Agaricus bisporus*). *Journal of the Science of Food and Agriculture* 26, 835–842.

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry* 48, 115–146.

Hanson, P.J., Todd Jr., D.E., Amthor, J.S., 2001. A six-year study of sapling and large-tree growth and mortality responses to natural and induced variability in precipitation and throughfall. *Tree Physiology* 21, 345–358.

Hart, S.C., Gehring, C.A., Selmants, P.C., Deckert, R.J., 2006. Carbon and nitrogen elemental and isotopic patterns in macrofungal sporocarps and trees in semiarid forests of the south-western USA. *Functional Ecology* 20, 42–51.

Hasselquist, N.J., Metcalfe, D.B., Högberg, P., 2012. Contrasting effects of low and high nitrogen additions on soil CO_2 flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. *Global Change Biology* 18, 3596–3605.

Heinemeyer, A., Ineson, P., Ostle, N., Fitter, A.H., 2006. Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows a strong dependence on recent photosynthates and acclimation to temperature. *New Phytologist* 171, 159–170.

Heinemeyer, A., Hartley, I.P., Evans, S.P., Carreira de la Fuente, J.A., Ineson, P., 2007. Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology* 13, 1786–1797.

Heinemeyer, A., Di Bene, C., Lloyd, A.R., Tortorella, D., Baxter, R., Huntley, B., Gelsomino, A., Ineson, P., 2011. Soil respiration: implications of the plant-soil continuum and respiration chamber collar-insertion depth on measurement and modeling of soil CO_2 efflux rates in three ecosystems. *European Journal of Soil Science* 62, 82–94.

Heinemeyer, A., Wilkinson, M., Vargas, R., Subke, J.-A., Casella, E., Morison, J.I.L., Ineson, P., 2012. Exploring the "overflow tap" theory: linking forest soil CO_2 fluxes and individual mycorrhizosphere components to photosynthesis. *Biogeosciences* 9, 79–95.

Hobbie, E.A., Weber, N.S., Trappe, J.M., 2001. Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence. *New Phytologist* 150, 601–610.

Hobbie, E.A., 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology* 87, 563–569.

Högberg, P., Plamboeck, A.H., Taylor, A.F.S., Fransson, P.M.A., 1999. Natural ^{13}C abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. *Proceedings of the National Academy of Sciences* 96, 8534–8539.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411, 789–792.

Högberg, M.N., Högberg, P., 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass, and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist* 154, 791–795.

Karnosky, D.F., 2003. Impacts of elevated atmospheric CO_2 on forest trees and forest ecosystems: knowledge gaps. *Environment International* 29, 161–169.

Karnosky, D.F., Zak, D.R., Pregitzer, K.S., Awmack, C.S., Bockheim, J.G., Dickson, R.E., Hendrey, G.R., Host, G.E., King, J.S., Kopper, B.J., Kruger, E.L., Kubiske, M.E., Lindroth, R.L., Mattson, W.J., McDonald, E.P., Noormets, A., Oksanen, E., Parsons, W.F.J., Percy, K.E., Podila, G.K., Riemenschneider, D.E., Sharma, P., Thakur, R., Sôber, A., Sôber, J., Jones, W.S., Anttonen, S., Vapaavuori, E., Mankovska, B., Heilman, W., Isebrands, J.G., 2003. Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Functional Ecology* 17, 289–304.

Karnosky, D.F., Pregitzer, K.S., Zak, D.R., Kubiske, M.E., Hendrey, G.R., Weinstein, D., Nosal, M., Percy, K.E., 2005. Scaling ozone responses of forest trees to the ecosystem level in a changing climate. *Plant, Cell and Environment* 28, 965–981.

Kasurinen, A., Helmisaari, H.S., Holopainen, T., 1999. The influence of elevated CO_2 and O_3 on fine roots and mycorrhizas of naturally growing young Scots pine trees during three exposure years. *Global Change Biology* 5, 771–780.

Kasurinen, A., Keinänen, M.M., Kaipainen, S.K., Nilsson, L., Vapaavuori, E., Kontro, M.H., Holopainen, T., 2005. Below-ground responses of silver birch trees exposed to elevated CO_2 and O_3 levels during three growing seasons. *Global Change Biology* 11, 1167–1179.

Kätterer, T., Reichstein, M., Andrén, O., Lomander, A., 1998. Temperature dependence of organic matter decomposition: a critical review using literature data analyzed with different models. *Biology and Fertility of Soils* 27, 258–262.

King, J.S., Pregitzer, K.S., Zak, D.R., Sober, J., Isebrands, J.G., Dickson, R.E., Hendrey, G.R., Karnosky, D.G., 2001. Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO_2 and tropospheric O_3. *Oecologia* 128, 237–250.

Koch, N., Andersen, C.P., Raidl, S., Agerer, R., Matyssek, R., Grams, T.E.E., 2007. Temperature-respiration relationships differ in mycorrhizal and non-mycorrhizal root systems of *Picea abies* (L.) Karst. *Plant Biology* 9, 545–549.

LI-COR, 2005. LI-8100 Automated Soil CO_2 Flux System Instruction Manual. LI-COR Biosciences, Lincoln, Nebraska.

Lilleskov, E.A., Bruns, T.D., Dawson, T.E., Camacho, F.J., 2009. Water sources and controls on water-loss rates of epigeous ectomycorrhizal fungal sporocarps during summer drought. *New Phytologist* 182, 483–494.

Lloyd, J., Taylor, J.A., 1994. On the temperature dependence of soil respiration. *Functional Ecology* 8, 315–323.

López-Gutiérrez, J.C., Malcolm, G.M., Koide, R.T., Eissenstat, D.M., 2008. Ectomycorrhizal fungi from Alaska and Pennsylvania: adaptation of mycelial respiratory response to temperature? *New Phytologist* 180, 741–744.

Loya, W.M., Pregitzer, K.S., Karberg, N.J., King, J.S., Giardina, C.P., 2003. Reduction of soil carbon formation by tropospheric ozone under increased carbon dioxide levels. *Nature* 425, 705–707.

Malcolm, G.M., López-Gutiérrez, J.C., Koide, R.T., Eissenstat, D.M., 2008. Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi. *Global Change Biology* 14, 1169–1180.

Malcolm, G.M., López-Gutiérrez, J.C., Koide, R.T., Eissenstat, D.M., 2009. Corrigendum: acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi. *Global Change Biology* 15, 2333.

Miller, R.M., Fitzsimmons, M.S., 2011. Fungal growth in soils. In: Ritz, K., Young, I. (Eds.), *The Architecture and Biology of Soils*. CAB International, Cambridge, MA, pp. 149–163.
Parrent, J.L., Morris, W.F., Vilgalys, R., 2006. CO_2-enrichment and nutrient availability alter ectomycorrhizal fungal communities. *Ecology* 87, 2278–2287.
Parrent, J.L., Vilgalys, R., 2007. Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO_2 and nitrogen fertilization. *New Phytologist* 176, 164–174.
Parrent, J.L., Vilgalys, R., 2009. Expression of genes involved in symbiotic carbon and nitrogen transport in *Pinus taeda* mycorrhizal roots exposed to CO_2 enrichment and nitrogen fertilization. *Mycorrhiza* 19, 469–479.
Philip, J.R., 1966. Plant water relations: some physical aspects. *Annual Review of Plant Physiology* 17, 245–268.
Pickles, B.J., Egger, K.N., Massicotte, H.B., Green, D.S., 2012. Ectomycorrhizas and climate change. *Fungal Ecology* 5, 73–84.
Podila, G.K., Miller, R.M., Cseke, L.J., White, H.L., Allison, V., 2011. Ecogenomics of mycorrhizal interactions mediated by the aspen genome under elevated ozone and carbon dioxide. In: Joshi, C.P., DiFazio, S.P., Kole, C. (Eds.), *Genetics, Genomics and Breeding of Poplar*. Science Publishers, Enfield, New Hampshire, USA, pp. 327–352.
Prasad, A.R.S., Kurup, C.K.R., Maheshwari, R., 1979. Effect of temperature on respiration of a mesophilic and a thermophilic fungus. *Plant Physiology* 64, 347–348.
Pregitzer, K.S., King, J.S., Burton, A.J., Shannon, E.B., 2000. Responses of tree fine roots to temperature. *New Phytologist* 147, 105–115.
Pregitzer, K.S., Burton, A.J., King, J.S., Zak, D.R., 2008. Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3. *New Phytologist* 180, 153–161.
Rivera, C.S., Blanco, D., Salvador, M.L., Venturini, M.E., 2010. Shelf-life extension of fresh *Tuber aestivum* and *Tuber melanosporum* truffles by modified atmospheric packaging with microperforated films. *Journal of Food Science* 75, 225–233.
Russell, C.P., Voroney, R.P., 1998. Carbon dioxide efflux from the floor of a boreal aspen forest. I. Relationship to environmental variables and estimates of C respired. *Canadian Journal of Soil Science* 78, 301–310.
Rygiewicz, P.T., Andersen, C.P., 1994. Mycorrhizae alter quality and quantity of carbon allocated below ground. *Nature* 369, 58–60.
Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. *Biogeochemistry* 48, 7–20.
Schlesinger, W., Lichter, J., 2001. Limited carbon storage in soil and litter of experimental forest plots under elevated atmospheric CO_2. *Nature* 411, 466–469.
Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis, third ed. Academic Press (Elsevier Ltd.), London, UK, pp. 316–319.
Souto, C., Pellissier, F., Chiapusio, G., 2000. Allelopathic effects of humus phenolics on growth and respiration of mycorrhizal fungi. *Journal of Chemical Ecology* 26, 2015–2023.
Staddon, P.L., 2005. Mycorrhizal fungi and environmental change: the need for a mycocentric approach. *New Phytologist* 167, 635–637.
Treseder, K.K., Allen, M.F., 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. *New Phytologist* 147, 189–200.
Trocha, L.K., Mucha, J., Eissenstat, D.M., Reich, P.B., Oleksyn, J., 2010. Ectomycorrhizal identity determines respiration and concentrations of nitrogen and non-structural carbohydrates in root tips: a test using *Pinus sylvestris* and *Quercus robur* saplings. *Tree Physiology* 30, 648–654.
van Diepen, L.T.A., 2008. The Role and Diversity of Arbuscular Mycorrhizal Fungi in Acer Saccharum Dominated Forest Ecosystems under Natural and N-amended Conditions. PhD Thesis. School of Forest Resources and Environmental Sciences, Michigan Technological University, Houghton, Michigan.
van Diepen, L.T.A., Lilleskov, E.A., Pregitzer, K.S., Miller, R.M., 2010. Simulated nitrogen deposition causes a decline of intra- and extraradical abundance of arbuscular mycorrhizal fungi and changes in microbial community structure in northern hardwood forests. *Ecosystems* 13, 683–695.
Varoquaux, P., Gouble, B., Barron, C., Yildiz, F., 1999. Respiratory parameters and sugar catabolism of mushroom (*Agaricus bisporus* Lange). *Postharvest Biology and Technology* 16, 51–61.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, fourth ed. Springer-Verlag, New York, New York, USA.
Wallander, H., 1995. A new hypothesis to explain allocation of dry matter between mycorrhizal fungi and pine seedlings in relation to nutrient supply. *Plant and Soil* 168/169, 243–248.
Wallander, H., Nilsson, L.O., Hagerberg, D., Bååth, E., 2001. Estimation of the biomass and season growth of external mycelium of ectomycorrhizal fungi in the soil. *New Phytologist* 151, 753–760.
Wallander, H., Göransson, H., Rosengren, U., 2004. Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. *Ecosystem Ecology* 139, 89–97.
Wilkinson, A., Solan, M., Alexander, I., Johnson, D., 2012. Species richness and nitrogen supply regulate the productivity and respiration of ectomycorrhizal fungi in pure culture. *Fungal Ecology* 5, 211–222.
Wilson, J.M., Griffin, D.M., 1975. Water potential and the respiration of microorganisms in the soil. *Soil Biology and Biochemistry* 7, 199–204.
Winkler, J.P., Cherry, R.S., Schlesinger, W.H., 1996. The Q_{10} relationship of microbial respiration in a temperate forest soil. *Soil Biology & Biochemistry* 28, 1067–1072.
Yang, J., Chuankuan, W., 2006. Partitioning soil respiration of temperate forest ecosystems in northeastern China. *Acta Ecologica Sinica* 26, 1640–1647.
Zak, D.R., Holmes, W.E., Pregitzer, K.S., 2007. Atmospheric CO_2 and O_3 alter the flow of ^{15}N in developing forest ecosystems. *Ecology* 88, 2630–2639.