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REGULAR ARTICLE

Soil carbon and nitrogen mineralization following deposition of insect frass and greenfall from forests under elevated CO_2 and O_3

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Abstract Elevated CO_2 and O_3 alter tree quality and the quality of herbivore inputs, such as frass, to forest soil. Altered quality or quantity of herbivore inputs to the forest floor can have large impacts on belowground processes. We collected green leaves and frass from whitemarked tussock moth caterpillars from aspen-birch stands at the Aspen Free Air CO_2 Enrichment (FACE) site near Rhinelander, WI, USA. Small or large quantities of frass, greenfall, or a 1:1 ratio of frass and greenfall were added to microcosms for each FACE treatment (control, $+CO_2$, $+O_3$, $+CO_2+O_3$). We measured initial frass and greenfall quality, and recorded microbial respiration, and nitrate leaching over 40 days. Elevated carbon dioxide (eCO_2) and tropospheric ozone (eO_3) significantly altered the carbon, nitrogen, and condensed tannin content of insect frass and green leaves. Although FACE treatments affected input quality, they had minimal effect on microbial respiration and no effect on nitrogen leaching. In contrast, input quantity substantially influenced microbial respiration and nitrate leaching. Respiratory carbon loss and nitrate immobilization were nearly double in microcosms receiving large amounts of herbivore inputs than those receiving no herbivore inputs. Small amounts of herbivore inputs, however, did not significantly alter microbial respiration or immobilization, suggesting that effects of herbivore inputs on soil processes will be detected only at moderate to high herbivory/input levels. These results suggest that subtle changes in frass and greenfall quality may not affect soil nutrient cycling. In contrast, environmental change induced increases in insect population size or frass and greenfall inputs to the soil may substantially impact nutrient cycling.

Keywords Carbon and nitrogen mineralization · Aspen FACE · *Orgyia leucostigma* · Canopy herbivore inputs · Soil respiration · Carbon dioxide · Ozone

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Introduction

Forest ecosystems of the 21st century face a multitude of global change factors that will alter their structure and function, as well as the services they provide. Fragmentation, invasion of alien species and changes in the atmosphere will alter forests directly through effects on plants and indirectly through impacts on higher trophic levels (e.g., herbivores). Changes in atmospheric concentrations of two gases, carbon dioxide and tropospheric ozone, are expected to have significant impacts on forest ecosystems (Karnosky et al. 2003). Annual human-caused CO_2 emissions have increased more

than 80% since 1970 (IPCC 2007) and tropospheric ozone concentrations are expected to increase from an average ambient background concentration of 40 ppb today to 68 ppb by 2050 and 85 ppb by 2100 (Akimoto 2003; Wittig et al. 2009). Numerous studies have evaluated the effects of both elevated CO_2 (eCO_2) and elevated O_3 (eO_3) on tree health, nutrient status and defense characteristics. Generally, forest trees respond to eCO_2 with increased photosynthesis and growth above- and below-ground (Saxe et al. 1998; Karnosky et al. 2003), decreased foliar nitrogen and increased foliar carbohydrates and phenolics, resulting in increased carbon to nitrogen ratios (Zvereva and Kozlov 2006; Stiling and Cornelissen 2007; Lindroth 2010). Unlike the fertilizing effects of CO_2, tropospheric O_3 causes significant damage to plants, inducing foliar injury (Karnosky 1976; Karnosky et al. 2003) and decreasing photosynthesis and growth (Karnosky et al. 2003). Changes in phytochemistry of trees exposed to eO_3 can shift in magnitude or direction depending on the timing of the sampling (season), and the species and genotypes (O_3 sensitive vs. O_3 resistant) involved (Lindroth et al. 2001; Holton et al. 2003; Kopper and Lindroth 2003; Lindroth 2010). Although the effects of eCO_2 and eO_3 on plant quality vary, the implications are clear: eCO_2 and eO_3 alter the quality of forest trees for herbivores.

Changes in tree quality due to eCO_2 and eO_3 have been shown to affect herbivore growth, survivorship and abundance (Bezemer and Jones 1998; Zvereva and Kozlov 2006; Valkama et al. 2007; Lindroth 2010), but the implications for herbivore-mediated ecosystem processes have received little attention. Canopy herbivory can strongly affect nutrient cycling (Belovsky and Slade 2000; Schowalter 2000; Hunter 2001). Canopy insects may influence soil nutrient dynamics through four types of soil surface inputs: frass deposition, turnover of insect cadavers, changes in throughfall chemistry, or changes in quality or quantity of leaf litter that falls to the forest floor (e.g., greenfall deposition) (Hunter 2001). Frass and greenfall inputs, in particular, are important because they occur during the growing season and frass inputs have been shown to influence tree nutrient levels (Christenson et al. 2002; Frost and Hunter 2007) and soil carbon and nitrogen cycling within the same growing season (Frost and Hunter 2004, 2007). This fast processing of organic matter is possible because, unlike senesced leaves that have much of their nutrient content resorbed, frass and greenfall inputs are "higher quality" inputs that may contain 74% more available nitrogen than senescent leaves (Risely and Crossley 1993; Fonte and Schowalter 2004; Madritch et al. 2007a). Further, soil nitrogen inputs from frass can exceed those from leaf litter during severe outbreaks (Fogal and Slansky 1984; Grace 1986; Hollinger 1986). Frass and greenfall inputs to the soil may result in increased nitrogen mineralization (Constantinides and Fownes 1994; Lightfoot and Whitford 1990; Reynolds et al. 2000) leading to enhanced microbial immobilization (Lovett and Ruesink 1995; Christenson et al. 2002; Lovett et al. 2002) or export from the system (Swank and Crossley 1988; Eshleman et al. 1998). Nitrogen export may occur rapidly (e.g., within 1 week) if timed with a rainfall event, but in most situations the nitrogen is immobilized by microbes or fungi near the soil surface (top 0–30 cm) and is not available for plant uptake (Christenson et al. 2002; Lovett et al. 2002; Frost and Hunter 2007).

The effects of herbivore inputs on soil carbon and nitrogen dynamics depend on both input quality and quantity. Input quality may change due to the direct effects of atmospheric chemistry on foliar chemistry, which, in turn, affects the chemistry of both frass and greenfall. Knepp et al. (2007) demonstrated that under eCO_2 *Polyphemus* caterpillars have altered carbon and nitrogen concentrations and caterpillar frass has altered elemental composition, phenolic content and carbon to nitrogen ratios. Input quantity may change due to the effects of foliar chemistry on the ingestion and egestion rates of individual herbivores (Lindroth 1996), as well as on herbivore population density (Liebhold and Elkinton 1988). Of these, changes in population densities are likely particularly important, as research has documented increased effects of herbivore inputs at higher population densities (Hunter et al. 2003).

Given that atmospheres of the future are likely to alter the quality and quantity of frass and greenfall inputs, we investigated how eCO_2 and eO_3 would affect carbon respiration of soil microorganisms and soil nitrate leaching. We employed an experimental microcosm approach, using different quantities of whitemarked tussock moth (*Orgyia leucostigma* Fitch) frass and greenfall, both of which varied in quality due to atmospheric chemistry treatments administered at the Aspen Free Air CO_2 Enrichment (FACE) facility near Rhinelander, WI, USA.

Methods

Aspen FACE facility

The Aspen FACE site consists of 12 circular plots (rings), each 30 m in diameter. During the growing season, each ring receives one of two levels of CO_2 (ambient ~350 ppm, aCO_2; elevated ~550 ppm) and O_3 (ambient ~35 ppb, aO_3; elevated ~50 ppb). Thus, at the level of atmospheric gas treatment, this is a two-way factorial experiment with three replicates of each of the four treatment combinations. Fumigation of trees began in 1998 with eCO_2 and eO_3 dispensed at concentrations predicted for 2060. Elevated O_3 is fumigated at approximately 1.5 times that of monitored, daily, ambient levels (Dickson et al. 2000).

Frass and greenfall

In August 2006, we reared whitemarked tussock moth larvae from eggs obtained from the Canadian Forest Service (Sault St. Marie, ON, Canada), at 22°C in a Percival® growth chamber. The whitemarked tussock moth was chosen because it is a common generalist, native herbivore in this system. This tussock moth is not considered an outbreak species, but has similar feeding behavior and gut and frass chemistry to the gypsy moth (*Lymantria dispar* L.) (Kopper et al. 2002; Lovett et al. 1998; Frost and Hunter 2007). Larvae were fed paper birch foliage from trees at Aspen FACE that were not exposed to the fumigation treatments. Once larvae had reached the third instar, 10 individuals were put into a mesh enclosure on each of four aspen (*Populus tremuloides* Michx., clone 216) and four birch (*Betula papyrifera* Marsh.) trees in each of the 12 rings. Larvae were then allowed to feed until pupation. Frass was collected from fifth-instar caterpillars, air-dried, and weighed. Equal amounts (1:1 mix by dry mass) of frass from aspen- and birch-fed caterpillars were then combined into a single aggregate pool for each FACE ring (three replicate FACE rings per fumigation treatment). "Greenfall" was artificially generated for this study using green leaves collected from each FACE ring in August 2006. To preserve chemical characteristics, field collected leaves were stored on crushed ice while in the field, flash frozen in liquid nitrogen, and freeze-dried. Freeze-dried leaves easily crumbled into small pieces. We selected pieces roughly 0.1 to 0.5 cm^2 for this experiment. Equal amounts of aspen and birch foliage were weighed and pooled (1:1 mix by dry mass) for each FACE ring.

Subsamples of frass and composite greenfall were measured for carbon and nitrogen concentration (% dry mass) with a Thermo Finnigan (San Jose, CA, USA) Flash 1112 elemental analyzer. Condensed tannins of frass and greenfall were extracted with 70% acetone at 4°C and quantified by the acid butanol method of Porter et al. (1986), with a purified 1:1 mix of aspen and birch tannin standards. We do not report lignin concentrations in this study because the fine particulate nature of frass caused too much loss of material during the standard lignin digestion procedure.

Microcosms

We set up seven microcosms for each of the 12 FACE rings. Microcosms consisted of pieces of clear acrylic plastic (18 cm in height and 6 cm in diameter) enclosed at the bottom by 1 mm nylon mesh and a clear plastic funnel. Each microcosm was filled to 10 cm depth with 150 g (dry mass) of field moist soil mix. This mix contained 100 g of topsoil from a non-fumigated portion of the Aspen FACE site and 50 g of sand, added to improve drainage. On top of the soil, we placed 1.5 g of chopped, air-dried leaf litter to simulate natural soil conditions at Aspen FACE. This litter was also collected from a non-fumigated portion of the FACE facility during August 2006, and was a mixture of partially decomposed aspen and birch leaves from previous growing seasons. Soil and old leaf litter were defaunated by repeated (three times) freezing and thawing. Sand was sterilized using an autoclave.

After adding soil and old leaf litter, each microcosm was flushed with 30 ml of double distilled water to rehydrate the soil and litter. After four days, either a low or high quantity of frass, greenfall, or a mix of frass and greenfall (1:1 mix by dry mass) was added to each microcosm (Table 1). Input quantities were based on estimates of gypsy moth herbivory in mature aspen stands calculated from aspen specific leaf area, leaf area index, litterfall and gypsy moth digestion efficiencies (Raich and Nadelhoffer 1989; Lindroth and Hwang 1996; Steele et al. 1997; Roth et al. 1998; Burrows et al. 2002; Davidson et al. 2002; Madritch et al. 2007a). The 100 mg treatments represent frass and greenfall inputs from background canopy herbivory of less than 20% leaf area consumed while the

Table 1 Frass and greenfall treatments applied to the soil microcosms. Each of the seven treatments was applied to one microcosm for each of the 12 FACE rings for a total of 84 microcosms

	Treatment	Abbreviation	Quantity (mg)
1	Control	C	0
2	Frass (low)	FL	100
3	Greenfall (low)	GL	100
4	Frass + Greenfall (low)	FGL	50+50
5	Frass (high)	FH	700
6	Greenfall (high)	GH	700
7	Frass + Greenfall (high)	FGH	350+350

700 mg treatments represent frass and greenfall inputs from outbreak herbivory levels of greater than 70% leaf area consumed. After substrates were added to the microcosms, we added 20 ml of distilled water to rehydrate the inputs. Microcosms were incubated at room temperature (19°C) for 40 days.

Soil carbon and nitrogen mineralization

On days 1–5, 7, 9, 11, 16, 18, 21, 24, 30, and 37 we measured CO_2 efflux from each of the 84 microcosms (12 rings × 7 microcosms) for two minutes using a PP Systems (Amesbury, MA) infrared gas analyzer. Five milliliters of water were added to each microcosm approximately 24 h before each CO_2 measurement and as necessary between measurements to maintain soil moisture levels. The amount of water added to each microcosm over the entirety of the study approximated the average summer monthly rainfall per 37 days in northern Wisconsin. We report CO_2 respired in conventional units of $g/m^2/hr$.

After the 40 day incubation period, microcosms were flushed with 50 ml distilled water. Leachate was collected by drawing a vacuum through each microcosm and was analyzed for combined NO_2 and NO_3 concentration using a modified version of the vanadium chloride method (Miranda et al. 2001; Doane and Horwath 2003; Allison et al. 2008). Leachate was analyzed for NH_4 concentration using a modified version of the Berthelot-salicylate method (Weatherburn 1967; Madritch et al. 2007a; Allison et al. 2008). Ammonium concentration is typically much lower than nitrate concentration in leachate due to lower mobility. Nitrogen concentration of leachate is reported as NO_3-N and NH_4-N loss (mg/L) per liter of leachate.

Statistical analysis

Average carbon, nitrogen, and condensed tannins in frass and greenfall pools from the 12 FACE rings were analyzed with a blocked, two-way ANOVA, where effects were block, CO_2, O_3, and $CO_2 \times O_3$ interaction. CO_2 respiration and nitrogen leaching results were analyzed using a blocked, split-plot ANOVA. Whole plot effects were related to input quality and included experimental block, CO_2, O_3, and $CO_2 \times O_3$ interaction. At the sub-plot level, effects were microcosm treatment (Table 1), $CO_2 \times$ microcosm treatment, $O_3 \times$ microcosm treatment, and $CO_2 \times O_3 \times$ microcosm treatment. Correlation analysis was used to reveal the pattern of relationship between nitrate leaching and soil respiration. Analyses were conducted using JMP® 8.0.1 software (SAS Institute, Inc, Cary, NC, USA). P-values are considered statistically significant at $\alpha \leq 0.05$ and marginally significant for $0.05 < \alpha < 0.1$ (Filion et al. 2000).

Results

Input quality

FACE treatments significantly affected frass quality (Table 2). Frass carbon concentration was 1% lower, nitrogen concentration was unaltered, and condensed tannin concentration was 41% higher in eCO_2 plots compared with aCO_2 plots. Frass carbon concentration was not affected by eO_3, but nitrogen concentration was reduced 12% and condensed tannin concentration was increased 30% at eO_3 compared with aO_3. However, the 30% increase in tannins at eO_3 was not statistically significant.

Elevated CO_2 and O_3 also affected green leaf quality (Table 2). Greenfall carbon concentration was 1% lower in eCO_2 plots compared with aCO_2 plots. Greenfall nitrogen and condensed tannin concentrations were not altered by eCO_2. Elevated O_3 increased greenfall carbon concentration 1%. Greenfall nitrogen concentration decreased 9% and condensed tannin concentration increased 13% compared with aO_3 but the decrease in tannins was not significant. For comparison, greenfall, frass, and leaf litter collected

Table 2 Mean (±1 SE) percent carbon, nitrogen, and condensed tannins of frass and greenfall added to microcosms. Statistics represent the results of two-way crossed ANOVA tests (df 1,6). Significant P-values (<0.10) are indicated in bold

	Control	+CO_2	+O_3	+CO_2+O_3	CO_2		O_3		$CO_2 \times O_3$	
					F	*P*	*F*	*P*	*F*	*P*
Frass carbon	50.21±0.25	49.61±0.16	50.24±0.09	49.61±0.21	10.52	**0.02**	0.01	0.94	0.01	0.95
Frass nitrogen	1.18±0.07	1.12±0.06	0.99±0.04	1.05±0.05	0.00	0.99	5.34	**0.06**	1.12	0.33
Frass tannins	6.83±0.62	11.32±1.53	10.49±2.20	12.93±1.96	4.00	**0.09**	2.33	0.17	0.38	0.56
Greenfall carbon	49.95±0.11	49.5±0.11	50.36±0.07	50.1±0.23	4.91	**0.07**	10.23	**0.02**	0.39	0.56
Greenfall nitrogen	1.87±0.02	1.88±0.04	1.59±0.04	1.83±0.13	2.77	0.15	5.51	**0.06**	2.48	0.17
Greenfall tannins	17.32±2.37	20.01±3.34	23.44±4.43	18.82±2.53	0.08	0.79	0.5	0.51	1.09	0.34

from the FACE rings had carbon concentrations of 50%, 50%, and 49%, respectively, and nitrogen concentrations of 1.8%, 1.0%, and 0.9%, respectively.

Soil processes

Consistent with the minimal effects of FACE CO_2 and O_3 treatments on input quality, we found only minor effects on microbial respiration (Table 3; Fig. 1a, c). We found no CO_2 or O_3 main effects on microbial respiration averaged across microcosm treatments.

Table 3 Two-way crossed split-plot ANOVA of microbial respiration and nitrate leaching from microcosms. P-values in bold are significant at $\alpha \leq 0.05$. Microcosm trt. (= treatment) includes the seven microcosm treatments from Table 1

Source	*F*	df	*P*
Microbial respiration			
CO_2	1.92	1,6	0.21
O_3	1.04	1,6	0.35
$CO_2 \times O_3$	0.99	1,6	0.36
Microcosm trt.	21.57	6,48	**<0.01**
CO_2 × microcosm trt.	2.05	6,48	0.08
O_3 × microcosm trt.	0.30	6,48	0.93
$CO_2 \times O_3$ × microcosm trt.	1.91	6,48	0.10
Nitrate-N leaching			
CO_2	0.53	1,6	0.50
O_3	0.14	1,6	0.72
$CO_2 \times O_3$	0.10	1,6	0.77
Microcosm trt.	6.40	6,48	**<0.01**
CO_2 × microcosm trt.	0.92	6,48	0.49
O_3 × microcosm trt.	1.60	6,48	0.17
$CO_2 \times O_3$ × microcosm trt.	0.90	6,48	0.50

Microbial respiration was marginally affected by the interaction of CO_2 and microcosm treatment ($F_{6,48}$ = 2.05, P=0.08). We found a trend for larger differences in CO_2 respiration between low and high inputs at eCO_2 compared with aCO_2. Microcosms with the FGL treatment had greater carbon respiration than control microcosms at aCO_2 but similar carbon respiration to FGL microcosms at eCO_2. Microbial respiration was not significantly different between greenfall, frass, and combinations of the two at the same quantity of input (Fig. 1b, c) even though the substrates differed slightly in terms of carbon and nitrogen concentrations.

In contrast to input quality, input quantity had a substantial positive effect on microbial respiration (Table 3, Fig. 1b, c). Losses from microcosms receiving small amounts of herbivore inputs were larger (0.079±0.006 g $CO_2/m^2/hr$) but not significantly different from those receiving no herbivore inputs (0.066±0.004 g $CO_2/m^2/hr$; Fig. 1b). However, respiratory carbon losses from microcosms receiving large amounts of herbivore inputs were nearly double (0.126±0.007 g $CO_2/m^2/hr$) those receiving no herbivore inputs (Table 3, Fig. 1b).

Nitrate loss from microcosms was unaffected by the CO_2 or O_3 treatments (Table 3, Fig. 2b, c) but was negatively related to input quantity. Nitrate concentrations of leachate from microcosms receiving small amounts of herbivore inputs was lower, but not significantly different from those receiving no herbivore inputs (72.5±8.9 vs. 80.3±6.9 mg/L; Fig. 2b). Nitrate concentrations of leachate from microcosms receiving large amounts of herbivore inputs were approximately half (38.5±8.0 mg/L) those receiving low herbivore inputs. However, only GH and FGH

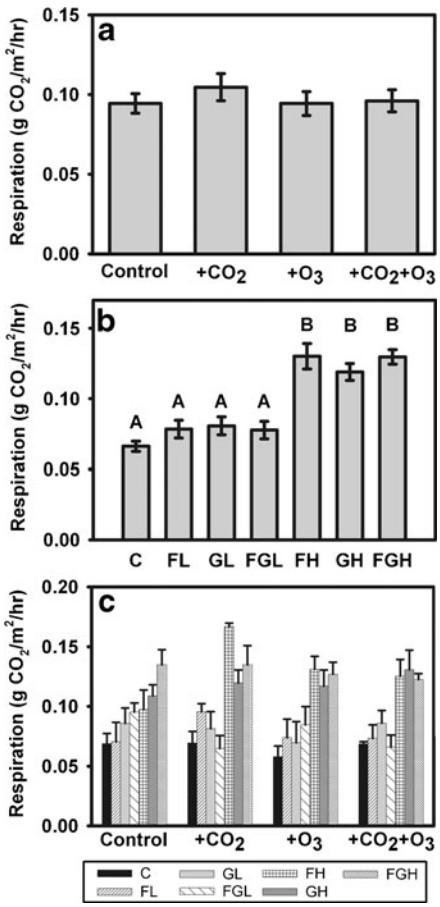

Fig. 1 Effects of FACE fumigation treatments (CO_2, O_3) and organic inputs (frass, greenfall) on soil respiration (±1 SE). ANOVA results are presented in Table 3. **a** effects of fumigation treatments, pooled across microcosm treatments. **b** effects of microcosm treatment, pooled across fumigation treatments. **c** effects of individual fumigation and microcosm treatments. Abbreviations for microcosm treatments are provided in Table 1

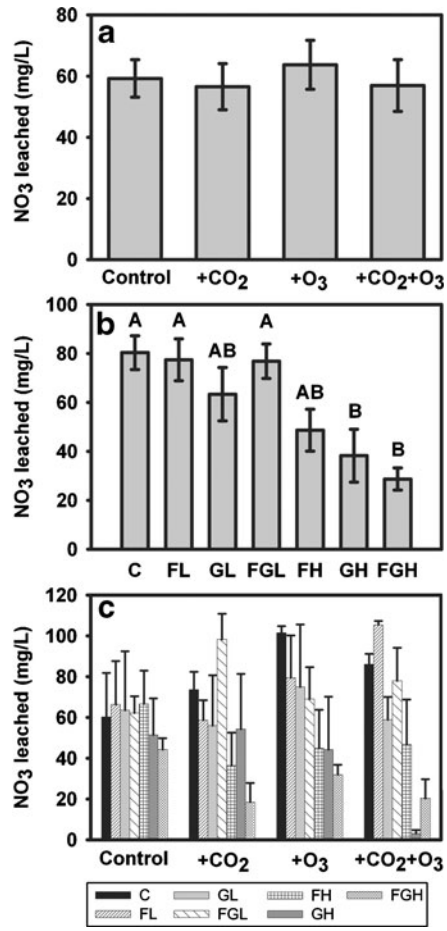

Fig. 2 Effects of FACE fumigation treatments (CO_2, O_3) and organic inputs (frass, greenfall) on nitrate concentration of leachate (±1 SE). ANOVA results are presented in Table 3. **a** effects of fumigation treatments, pooled across microcosm treatments. **b** effects of microcosm treatment, pooled across fumigation treatments. **c** effects of individual fumigation and microcosm treatments. Abbreviations for microcosm treatments are provided in Table 1

inputs (not FH) were significantly different from no input (Table 3, Fig. 2b). We found a trend for a greater reduction in nitrate leaching in microcosms with greenfall compared with frass organic inputs across fumigation treatments (Fig. 2b, c). We also found a clear negative correlation between respiratory carbon loss and nitrate leaching across microcosm treatments (Fig. 3).

Relatively little ammonium nitrogen leached from the soil microcosms. The average concentration of NH_4-N in leachate across all microcosms was 0.058± 0.003 mg/L, only 1% of the concentration of nitrate. Neither input quality nor quantity influenced rates of ammonium leaching.

Discussion

Effects of input quality and quantity on soil processes

Changes in frass and greenfall quality due to changes in atmospheric chemistry were sufficiently subtle that they did not significantly affect carbon and nitrogen mineralization. Differences in input quality, in terms of whether it was frass or greenfall, also had minor effects on soil carbon and nitrogen mineralization. In contrast, variation in the quantity of herbivore inputs strongly influenced carbon and nitrogen mineralization rates.

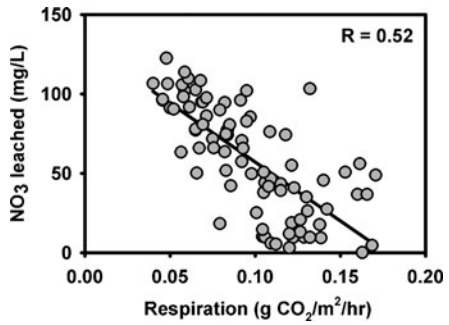

Fig. 3 Correlation of microbial respiration and nitrate leached for the 84 soil microcosms. Each point represents one microcosm

Elevated CO_2 and O_3 had relatively minor effects on frass and greenfall carbon and nitrogen concentrations. Condensed tannin levels increased in leaves exposed to eCO_2 and eO_3. Caterpillar frass also had increased amounts of tannins at eCO_2 and eO_3. Previous work with gypsy moth and forest tent caterpillars found that frass carbon to nitrogen ratios and condensed tannin levels reflected the carbon to nitrogen ratio and condensed tannin levels of the aspen foliage on which they fed (Madritch et al. 2007a). Frass of whitemarked tussock moth caterpillars fed birch condensed tannins also reflected the concentration of tannins in their food source (Kopper et al. 2002). These results suggest that if eCO_2 or eO_3 alter host plant chemical composition, frass chemical composition will be similarly altered. Altered chemical composition of frass, especially altered condensed tannin levels, could significantly alter soil processes because condensed tannins have been shown to reduce nutrient availability and slow leaf litter decomposition (Schweitzer et al. 2008) and alter soil respiration (Madritch et al. 2007b). Elevated CO_2 has been shown to reduce leaf litter quality (e.g., increased tannin concentrations and carbon to nitrogen ratios) at Aspen FACE, resulting in slower decomposition (Parsons et al. 2004, 2008). Similarly, lower frass or greenfall quality could impact soil processes, although the mostly weak effects of eCO_2 and eO_3 on input quality in this study were insufficient to do so.

Our results show that low levels of herbivore inputs (inputs from <20% leaf area removed) had a minor effect on soil microbial respiration, but high levels of herbivore inputs (inputs from >70% leaf area removed) nearly doubled microbial respiration. Previous studies suggest that small input levels can significantly alter soil processes, but do so less often than when inputs are large (e.g., during outbreaks) (Hunter et al. 2003). Only moderate or high input levels may provide enough labile carbon to allow detection of increased soil respiration. Both our results and those of Lovett and Ruesink (1995) support the concept that soil microbes do not respond unless frass inputs reach some carbon threshold. However, the response at even high input levels is likely to be short-lived (Frost and Hunter 2004). Soil temperature and precipitation events likely set the stage for the strength of the effect of herbivore inputs on soil microbe respiration rates. For example, high humidity or precipitation leaches frass carbon and nitrogen into the soil, while high temperature will increase microbial activity once inputs become available (Reynolds and Hunter 2001). Although we focused on microbial respiration, we recognize that part of the respiration we measured could be due to the frass microbial community and not the soil microbial community. We also recognize that the inclusion of leaf litter in our microcosms may have resulted in an early flush of CO_2 that masked smaller fumigation treatment effects on respiration.

Variation in input quantity also strongly influenced nitrogen leaching rates. Nitrate leaching was relatively high at low-input levels, while microbial immobilization occurred at high-input levels. Frost and Hunter (2007) suggest that significant amounts of frass nitrogen may be leached if frass inputs are timed with precipitation, but otherwise the nitrogen will be incorporated into soil organic matter. Indeed, frass nitrogen may rapidly move into nonexchangeable soil pools near the soil surface (top 30 cm) (Christenson et al. 2002). It appears, therefore, that leaching losses likely occur shortly after deposition but most frass nitrogen is rapidly immobilized (Frost and Hunter 2007). Our results add that the quantity of organic input may alter whether nitrate is leached from, or immobilized in, the soil. The clear negative correlation we found between respiratory carbon loss and nitrate leaching across microcosm treatments strongly supports the concept that only moderate to high carbon inputs supply enough labile carbon for soil microbial populations to grow and increase nitrate immobilization (Fig. 3). Regardless, frass nitrogen inputs change the timing of nitrogen input to the forest floor and can alter the ultimate distribution of

nitrogen, even within the season of defoliation (Frost and Hunter 2004). For example, Frost and Hunter (2007) found that nitrogen from frass deposited early in the summer was taken up by plants within the same growing season. Approximately 1.2% of the frass nitrogen ended up in senescent leaves of the same trees at the end of the growing season, linking the fast (frass) and slow (senescent leaves) nutrient cycles. However, plants may acquire senesced leaf nitrogen more easily than frass nitrogen (Christenson et al. 2002).

Greenfall, respiration, and nitrate leaching

Our results suggest that, similar to large frass inputs, microbial respiration is enhanced by large inputs of greenfall. In another study, Reynolds and Hunter (2001) found reduced microbial respiration in forest soils where greenfall was excluded. While soil respiration was similar after frass and greenfall inputs, nitrate immobilization appeared to be slightly, although not significantly greater, for greenfall than for frass. The 65% higher nitrogen concentration of greenfall compared with frass in our study may have afforded greater microbial population growth and, thereby, increased immobilization. Reynolds and Hunter (2001) found no leaching of nitrate in their greenfall exclusion plots and suggested this may have been due to immobilization by roots or microbes. ^{15}N isotope studies have been useful for tracking frass N cycling and should be performed for greenfall given its high resource quality for microbes. However, as for frass, greenfall effects in the soil may be short-lived and dependent on temperature and precipitation.

Limitations

Microcosm studies are useful because they reduce environmental complexity and facilitate interpretation of results, but they are also less ecologically realistic (Carpenter 1996; Verhoef 1996). For example, our microcosms did not include detritivores or plant roots, and both will have important effects on soil respiration and nitrogen dynamics. Also, we did not consider other herbivore inputs, such as throughfall, which can influence soil processes (Stadler et al. 2001, 2006). We also recognize that some nutrients may have leached from our frass in the rain storm that occurred the day we collected frass. However, no other rain events occurred during caterpillar rearing so very little leaching should have occurred. The nitrogen concentration of frass used in our study was approximately 1.0%, which is in line with values reported by Frost and Hunter (2007), but lower than the 2.4% documented by Lovett and Ruesink (1995) and the 1.8% reported by Madritch et al. (2007a). Our numbers may differ because results vary depending on the herbivore species used (Madritch et al. 2007a) and the time of sampling within a year. Insects feeding on early season foliage with greater concentrations of nitrogen and water and lower concentrations of defensive compounds (e.g., expanding leaves) could produce different results.

Implications of changes in herbivore input quantity

We found that soil processes were much more sensitive to the quantity of herbivore inputs than to the qualitative changes due to altered atmospheric chemistry. Liu et al. (2005) found that eCO_2 increased leaf litter inputs to the forest floor, and more recently that the increased inputs were more important to soil carbon and nitrogen cycles than were changes in litter chemistry due to eCO_2 (Liu et al. 2009). Larger inputs from the canopy also resulted in greater microbial respiration (King et al. 2004) and soil nitrogen concentration (Liu et al. 2009), in parallel with our large frass and greenfall inputs. These results suggest that altered atmospheric chemistry may have indirect effects on soil processes via changes in input quantity. Canopy damage rates at Aspen FACE have increased over 300% under eCO_2, resulting in a more than 40% increase in frass and greenfall (Couture, Meehan, and Lindroth unpublished data). Thus, continued increases in eCO_2 in the future may increase the quantity of herbivore inputs, which may substantially impact nutrient cycling in forest soils.

Summary

Elevated CO_2 and eO_3 affected frass and greenfall quality, but the changes were minimal, producing little effect on short-term microbial respiration and no effect on nitrogen leaching. In contrast, large quantities of herbivore inputs nearly doubled microbial respiration and nitrogen immobilization compared with no inputs. Although microbial response to herbivore inputs may be relatively short-lived, herbi-

vore inputs are very important for carbon and nitrogen cycling, at least within the season of deposition. Changes in the abundance or feeding habits of canopy herbivores due to environmental change could lead to changes in soil carbon sequestration and nitrogen balance in forests of the future.

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