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ORIGINAL PAPER

Diet influences rates of carbon and nitrogen mineralization from decomposing grasshopper frass and cadavers

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Abstract Insect herbivory can produce a pulse of mineral nitrogen (N) in soil from the decomposition of frass and cadavers. In this study, we examined how diet quality affects rates of N and carbon (C) mineralization from grasshopper frass and cadavers. Frass was collected from grasshoppers fed with natural or meridic diets which varied in N content. Frass was also collected from naturally foraging grasshoppers. Nitrogen concentration of frass was directly proportional to diet N, but N content of cadavers was not affected by diet. Incubations of soil plus frass were performed at constant soil moisture and temperature (15°C) for 28 days, after which levels of mineral N (KCl extract) were determined. About 44 % of C and N from the cadavers were mineralized after the 28-day incubation. Carbon mineralization of frass was not affected by diet or frass N but varied considerably among different food treatments: from 15 to 46 % of the C in frass was released as carbon dioxide. Generally, frass with C/N ratio greater than 20 resulted in net immobilization of N. Results suggest that much of the N in grasshopper frass and cadavers is labile and rapidly available for plants, depending on the quality of food consumed by the grasshoppers.

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Introduction

Herbivores can directly affect nitrogen (N) cycles by diverting N from plant tissues to the soil or indirectly through their effects on plant community composition and litter quality (Belovsky and Slade 2000; Cochran et al. 2000; Hunter 2001; Lovett et al. 2002). In some ecosystems, herbivores accelerate nutrient cycling (Schowalter et al. 1991; DeAngelis 1992; Seastedt and Crossley 1984; Ruess et al. 1989; Belovsky and Slade 2000; Chapman et al. 2003), while in others they decrease the rate of nutrient cycling (Pastor et al. 1993, 1998; Ritchie et al. 1998; Knops et al. 2000). Herbivory's indirect effects on N cycling depend in part on the herbivore's selection of plant species and on the responses of plants. Some plants may be tolerant of herbivory and respond by aggressive uptake of soil N and rapid regrowth, whereas loss of tissue in other plant species may lead to reduced ability to compete and thus a diminished role in the plant community (Bryant et al. 1983; Holland et al. 1992). Damage to plant tissues may induce chemical defenses, thereby reducing levels of herbivory and may also slow decomposition rate of plant litter (Findlay et al. 1996; Schimel et al. 1998; Hättenschwiler and Vitousek 2000).

The direct effects of herbivory on N cycling also depend in part on the amount of N in the excrement and how quickly it becomes available to plants. Nitrogen in intact tissues of perennial plants is largely recycled within the plant, being translocated to storage organs during the non-growing season. Similarly, annual plants at maturity will translocate N to seeds from other parts of the plant. Herbivores interrupt this internal cycling of N by consuming and

excreting N in plant tissues, diverting N from the plant to the soil. When plant tissue is cycled through herbivores, soil mineral N may be greater than in the absence of herbivory. Rapidly mineralized N may be lost from the ecosystem by leaching (Reynolds et al. 2000).

Mammalian herbivores have been studied extensively, but insect herbivores also have received considerable attention, primarily in forest ecosystems (Stadler et al. 2001; Reynolds and Hunter 2001; Lovett et al. 2002). Grasshoppers are ubiquitous herbivores of grasslands. During outbreaks, a large percentage of the N within plant tissues is consumed and ultimately transferred to the soil (Hewitt 1977; Quinn et al. 1993; Thompson and Gardner 1996). In cultivated crops, such as barley or wheat, invaded by grasshoppers preferentially feeding on broad-leaved weeds could result in a net transfer of N from the weeds to adjacent crop plants (Hinks et al. 1990; Fielding and Conn 2011).

Response of plants to defoliation depends in part on availability of N (Lovett et al. 2002). The rapidity with which N in insect frass becomes available could affect plants' ability to regrow after defoliation or alter competitive interactions between plant species. If rapidly mineralized, N may be available to plants in the same season aiding the recovery of damaged plants and favoring rapidly growing plant species. On the other hand, if the N is recalcitrant or immobilized, it will be more slowly available and may favor plants that are better competitors for soil N, those with mycorhizal associations, or are more efficient users of N.

Although frass and cadavers of herbivorous insects may alter the relative importance of N pathways in an ecosystem, especially during outbreak situations, few studies have examined rates of decomposition of these products. Lovett and Ruesink (1995) reported net immobilization of N in gypsy moth frass for 90 days. No studies have been conducted on effects of diet on frass quality and decomposition rates.

Grasshopper species common on grasslands encounter and consume a wide variety of plants with a wide range of nutritional quality. In the agroecosystems of Alaska, the mixed grass-and forb-feeding *Melanoplus borealis* and the grass-only feeder *Chorthippus curtipennis* are the dominant grasshopper species. Thus, these species were chosen as the subjects of our experiments. The objectives of this study were to evaluate the relationship between N concentration of grasshopper food and frass and rates of N and C mineralization. Knowledge of the effects of diet on frass quality and decomposition rates will increase our understanding of herbivore influence on nutrient cycles.

Materials and methods

Soil incubation experiments were conducted to determine how diet and subsequent frass N concentration influenced rates of N and C mineralization in grasshopper frass and cadavers. Grasshoppers were fed with meridic diets (defined diets consisting of a mix of natural and artificial ingredients) and natural plant foods of varying N concentrations. Because the grass-feeding *C. curtipennis* did not accept or thrive on the meridic diets, these treatments were limited to *M. borealis*. These species were collected at different times during the growing season and their frass collected. The frass from these treatments and cadavers from the meridic diet treatments were then analyzed for their N and C content and mineralization rates determined.

Frass collection and analysis

Meridic diets with five different N concentrations (Table 1) were fed to *M. borealis* grasshoppers which were reared in the lab. Casein was used to vary the diet N concentration, sucrose used as the carbohydrate supplement, and cellulose was used as non-nutritive filler. Other components (fatty acids, sterols, vitamins, and minerals) remained constant among diets. For a complete recipe, see Fielding and DeFoliart (2007). *M. borealis* grasshoppers were placed individually into plastic cages with meridic diet and a water source. Grasshoppers were fed these diets for about 20 days and, after discarding the frass from the first 3 days to allow grasshoppers to clear their digestive tracts of previously consumed foods, frass was collected and crushed with a mortar and pestle. Afterwards, the grasshoppers were frozen, and their cadavers were dried at 60°C for 2 days and then crushed. Samples of the meridic diets, frass, and cadavers were analyzed for N content using dry combustion (LECO TruSpec CN, LECO Corporation, St. Joseph, MI).

For the plant diets, grasshoppers were restricted to a single plant item and their frass collected. Plants were collected daily and grasshoppers were fed these items exclusively for 7–9 days in June and July (Table 1) and, after discarding frass from the first 3 days, the frass was collected and samples prepared as mentioned above. Plants fed to *M. borealis* included leaves of smooth bromegrass (*Bromus inermis* Leyss), barley (*Hordeum vulgare* L. subsp. *vulgare*), dandelion (*Taraxacum officinale* G. H. Weber ex Wiggers), and narrowleaf hawksbeard (*Crepis tectorum* L.). *C. curtipennis* was fed with leaves of bromegrass from the top of the plant (higher in N) and from the bottom of the plant (lower in N), barley, and rock sedge (*Carex saxatilis* L.). The barley was grown under typical farming methods including added fertilizer. The other plants were collected from unfertilized, fallow fields. A portion of the leaves from each day's collection were saved and dried for N analysis. Total N in the plant and frass samples was determined using dry combustion.

Frass was also collected from free-ranging grasshoppers collected in the Conservation Reserve Program fields in

Table 1 Concentration of N in grasshopper foods and frass with subsequent percentage of C and N mineralized after incubation in soil for 28 days at 15°C

Grasshopper sp.	Diet	Diet %N	Frass (or cadaver) %N	Frass C/N	% N mineralized[a]	% C mineralized
Mebo	Meridic	1.04	1.35	29.6	0	16
Mebo	Meridic	1.71	2.25	18.7	3	15
Mebo	Meridic	2.00	2.59	16.3	0	17
Mebo	Meridic	3.43	3.61	11.9	2	19
Mebo	Meridic	3.83	3.43	12.4	5	23
Mebo	Barley, June	5.88	5.30	6.8	57	–
Mebo	Barley, July	4.94	4.70	7.6	69	–
Mebo	Bromegrass, June	3.07	3.25	11.7	24	–
Mebo	Bromegrass, July	1.89	1.81	22.5	0	26
Mebo	Hawksbeard, June	2.54	1.19	31.1	0	19
Mebo	Hawksbeard, July	2.40	1.96	18.2	4	33
Mebo	Dandelion, June	3.82	1.65	22.6	0	46
Mebo	Dandelion, July	2.38	1.77	20.9	0	37
Chcu	Barley, July	5.33	4.60	8.0	59	25
Chcu	Bromegrass, June	2.54	2.14	13.3	1	44
Chcu	Bromegrass top, July	2.27	1.87	20.4	0	30
Chcu	Brome lower, July	1.51	1.55	24.0	0	32
Chcu	Sedge, July	0.95	1.09	32.9	0	25
Mebo	Free-range, June	n.a.	3.07	13.3	3	–
Mebo	Free-range, July	n.a.	3.35	11.3	5	25
Mebo	Free-range, August	n.a.	4.00	9.6	10	29
Chcu	Free-range, July	n.a.	3.06	12.7	7	–
Chcu	Free-range, August	n.a.	2.60	15.0	0	28
Cadavers	Meridic		10.7	4.3	44	44

Mebo Melanoplus borealis, *Chcu Chorthippus curtipennis*

[a] Mineral N = NH_4^+ + NO_3^-

Delta Junction, Alaska (145°20′50″ W, 63°56′28″ N). Grasshoppers (about 200 of each species) were collected in mid-afternoon using sweep nets and placed into separate plastic cages by species. They were left overnight to deposit their frass, and samples were processed and analyzed for C and N content as mentioned above.

Nitrogen mineralization determination

Soil used for the C and N mineralization incubations was obtained from the top 10 cm of an agricultural field at the University of Alaska Fairbanks Delta Junction Field Research Station. The soil was classified as a Volkmar silt loam, (coarse silty over sandy skeletal, mixed, superactive Aquic Eutrocryept). The soil was dried and sieved (<2 mm) to remove large roots and stones. The concentration of total C and N in the soil was 4.33 and 0.25 %, respectively. Organic matter concentration was 0.99 % and pH was 5.0. Field capacity of the soil was determined after suction was applied at 0.01 MPa for 3 h (Sparrow and Cochran 1988).

To determine the readily mineralizable pools of N, frass or cadavers were added to soil in 25-ml glass incubation jars with the lids fitted loosely on top to allow gas exchange. All frass and cadaver incubations contained 5.0 g of soil, 0.1 g of frass or 0.05 g of cadavers, and 1.32 ml distilled water (~90 % field capacity). A soil-alone treatment was used as a control (N=3). The frass and cadavers were added to the soil and mixed thoroughly before the distilled water was added. All treatments were incubated for 0 (baseline soil mineral N) and 4 weeks and consisted of three replicates. The jars that were incubated for 4 weeks were placed in a dark incubator set at 15°C, near the average growing season soil temperature in central Alaska. Deionized water was added as needed to maintain soil moisture at 90 % of field capacity. Short incubation times (14–28 days) are often used to evaluate the labile organic N and C pool (based on the N release curve) (Wang et al. 2004).

When the soil, frass, and cadaver treatments finished incubating, they were mixed thoroughly. One gram of soil (containing either the frass or cadaver amendment) was transferred into a plastic cup for KCl extraction. Ten milliliters of 2 N KCl was added to each cup, sealed with a cap,

and shaken in an Eberbach 6000 reciprocal shaker for 15 min at room temperature (22±1°C). The supernatant was decanted through a Whatman® No. 42 filter paper (pore size ~2.5 μm, Whatman®, Maidstone, England) and collected for analysis. Blank samples were acquired with the same procedure for extraction and filtration. Samples were stored temporarily in the dark at 4°C until chemical analysis could be performed. Samples did not stand for more than 2 days before analysis.

The concentration of mineral N (NH_4^+-N and NO_3^--N), expressed as micrograms of N per gram oven (105°C) dry soil + frass, in the extracts was analyzed colorimetrically with an automated rapid flow analyzer (RFA-305, Astoria Analyzer, Clackamas, OR). The N mineralization of the frass and cadavers in the soil was calculated by subtracting the total extractable NH_4^+-N and NO_3^--N for the soil-alone treatment from that of the frass and cadaver treatments. The net N mineralization was calculated as the difference in mineral N at time 0 and after 4 weeks of incubation.

Carbon mineralization (soil respiration) determination

To determine the potential C mineralization, we used the same frass and cadaver treatments as for N mineralization, except insufficient amounts of frass were obtained from some treatments and were omitted from the C mineralization experiment. These soil incubations consisted of 18 frass treatments, cadavers, and soil controls. All cadaver treatments and all but one frass treatments were replicated three times. Six replicates of the soil-only controls were made. Measurements were taken on days 1, 3, 5, 7, 10, 15, 19, 24, and 28 after the start of the experiment.

Each soil/substrate sample consisted of 5.0 g of soil, 1.32 ml distilled water, and either 0.1 g of frass or 0.05 g of cadavers. The soil/substrate samples were incubated under the same conditions as the N mineralization incubations.

Carbon mineralization was measured as carbon dioxide (CO_2) production using an infrared gas analyzer (Li-Cor Model 6252, Li-Cor, Lincoln, NE, USA) set at a flow rate of 150 ml/min. For CO_2 production measurement, incubation jars (with covers removed) were placed into a 200-ml airtight glass jar with tubing connected to the Li-Cor instrument such that air circulated continuously from the jars to the gas analyzer, through an air pump, and back through the sample jar. The samples were kept in a small incubator maintained at 15°C during measurements. Production of CO_2 was determined from the linear increase in CO_2 concentration during a 3-min measurement period (converted to parts per million per hour). Total volume of the system (jars, tubing, pump, and CO_2 analyzer) was determined by injecting a known amount of CO_2 and measuring the increase in CO_2 concentration. Using the total volume (184.3 ml), we converted the rate of increase of CO_2 into absolute amounts of C released (expressed as micrograms of C per gram air dry weight (AWD) of substrate per hour). We calculated net C mineralization as the difference in C mineralization between the soil-alone treatments and the amended soil treatments.

Statistical analysis

We tested for significant effects of diet N on frass total N or cadaver total N with linear regression. Analysis of covariance (Proc GLM, SAS Inst. 2003) was used to test whether the relationship between diet N and frass N differed among diet types (meridic, single plant items in lab, and wild grasshopper frass treatments) in *M. borealis*.

The amount of mineral N after 4 weeks incubation was log-transformed to stabilize variances and meet normality assumptions. Linear regression was used to relate the amount of N mineralized to frass N. Analysis of covariance (Proc GLM, SAS Inst. 2003) was used to examine the effects of diet type (meridic, single plant items in lab, and wild grasshopper frass treatments) on mineralization rates and whether the relationship between frass N content and N mineralization differed by diet type. The least significant difference test was employed to determine if there was a difference between the various treatments in regard to the amount of inorganic N (NH_4^+-N and NO_3^--N) produced. For all SAS procedures, significance was set at a probability level of 0.05.

Cumulative C mineralization was determined by calculating the area under the curve (trapezoidal method, MATLAB, The MathWorks, Natick, MA) of rates of C mineralization at 1–5 and 1–28 days. The 1 through 5 days interval captured peak rates of CO_2 production and the 1–28 days interval measured the total amount of C released during the incubation. This C mineralization data set met the assumptions of normality. Mineralized C was regressed against both diet N and frass N. The frass treatments were analyzed separately from the cadaver treatments because of the different nature of the inputs. Analysis of covariance was used to test for differences between grasshopper species and diet types (meridic diets vs. plants) and whether the effects of N concentration of the substrate on C mineralization rates differed by grasshopper species or diet type. If the relationship between N content and C mineralization differed between grasshopper species or food types, we would expect to find a significant interaction term between these effects and N content of the substrate. Because diet composition of the field-collected grasshoppers was unknown, differences in mineralization between dates of collection were tested for, instead of diet N.

Results

The N content of the meridic diets varied from 1.0 to 3.8 % (Table 1). Total N content of field-collected plants fed to *M.*

borealis and *C. curtipennis* ranged from 0.95 to 5.88 % N (Table 1). The highest levels of N (>4 %) were in barley leaves from a fertilized field. Other plants were from unfertilized, fallow fields and contained less than 4 % N. It was not possible to determine the N content of food consumed by field-collected grasshoppers.

Nitrogen concentration of frass collected from the various diet treatments ranged from 1.09 to 5.30 % N, and C/N ratios ranged from 32.9 to 6.8 (Table 1). In the analysis of covariance of the effects of grasshopper species and diet N on frass N concentration, the interaction between grasshopper species and food N content was not significant ($F_{1,14}$= 0.00, P=0.95) and so eliminated from the model. Grasshopper species had no effect on concentration of N in frass (Table 2), but N concentration in the diet strongly affected frass N concentration (Table 2).

Because *C. curtipennis* was fed only with grasses, this species was not included in analysis of covariance of the effect of diet type (meridic, grasses, or forbs) on frass N (Table 2). The interaction between diet type and food N concentration on frass N concentration for *M. borealis* was nonsignificant ($F_{2,7}$=2.8, P=0.13) and so was eliminated from the analysis of covariance (ANCOVA) model. Frass N concentration was affected by N concentration of their food, but diet type also affected the N concentration of frass (Table 2). The concentration of N in frass from grasshoppers feeding on forbs was lower for a given concentration of N in their diet than when feeding on meridic or grass diets. Least square means (% N of frass ± s.e.) were 1.9±0.19, 3.0±0.21, and 3.1±0.18 for forbs, grasses, and meridic diets, respectively (P<0.01 in each comparison with forbs). There was a positive, linear response of N concentration of frass to N concentration in the food (y=0.80x +0.27, r^2=0.77; P<0.001).

Table 2 Results of ANCOVA testing for effects of grasshopper species, diet type, and concentration of N in food or frass on concentration of N in grasshopper frass and C mineralization after 7 and 28 day incubation

ANCOVA source	SS	*df*	*F* ratio	*P* value
Concentration of N in grasshopper frass				
Food N	21.69	1	60.98	<0.0001
Grasshopper species	0.02	1	0.02	0.82
Error	27.76	15		
Concentration of N in grasshopper frass (*Melanoplus borealis*)				
Food N	9.77	1	66.42	<0.0001
Diet type	3.93	2	13.36	0.002
Error	1.32	9		
Cumulative C mineralized after 7 days				
Frass N	1.08×10^9	1	11.44	0.0054
Diet type	6.52×10^9	2	34.59	<0.0001
Error	7.86×10^9	12		
Cumulative C mineralized after 28 days				
Frass N	0.72×10^9	1	1.25	0.29
Diet type	7.08×10^9	2	6.17	0.014
Error	14.01×10^9	12		

Cadaver N was not affected by N content of the diets ($F_{1,9}$=0.01, P=0.92) and only varied from 10.49 to 10.71 % N. The N content of frass of *M. borealis* increased later in the season, from 3.07 % in June to 3.35 % in July, and 4.00 % in August, suggesting that foods relatively high in N were available throughout the season. Frass content of free-ranging *C. curtipennis* was 3.06 % N in July and 2.60 % N in August.

Log_e N mineralized after 4 weeks incubation was proportional to the N content of frass (y=1.51x−0.66, r^2=0.84; P< 0.001). Mean mineral N of soil alone was 12.6 μg C (g ADW)$^{-1}$ (±1.9 s.e.) at time 0 and after 28 days incubation was 28.5 μg C (g ADW)$^{-1}$ (±0.9 s.e.). In most of the diet treatments, immobilization of N occurred when frass N concentrations were less than 2.2–3.0 %, corresponding to C/N ratios greater than 20.

Grasshopper species had no effect on net N mineralized ($F_{1,20}$=1.14, P=0.16). Diet type also had no effect on net N mineralized ($F_{2,13}$=0.05, P=0.99). The frass with the highest net N mineralization was that from grasshoppers feeding on barley leaves. *M. borealis* and *C. curtipennis* produced frass with >4.5 % N, and C/N ratios less than 8 on this diet (Table 1). From 58 to 69 % of the N originally in the frass was available to plants after the 4-week incubation. Frass from free-ranging grasshoppers was less than 4.0 % N, with C/N ratios of 15 or less and resulted in positive net N mineralization, but only about 10 % or less of the N was mineralized within the 4-week period (Table 1). Two samples of frass from free-ranging *C. curtipennis* were collected, but only the frass from earlier in the season, containing 3.06 % N, resulted in net N mineralization (Table 1).

Some of the N contained in grasshopper cadavers was in mineral form at time 0 (105.9±4.3 ppm). After 4 weeks of incubation, cadavers resulted in a large amount of net N mineralized (mean 463.5 ppm±7.6 s.e.), representing 44 % of the N contained within the cadavers.

During the first week of the incubation, fungal hyphae covered many frass and cadaver samples but was not found in the soil-alone samples. Carbon dioxide accumulation peaked within the first 5–7 days in all frass and cadaver treatments and declined exponentially (Fig. 1).

Linear regressions of C released during the first 7 days, or the 28-day duration of the experiment, on frass N concentration were not significant ($F_{1,17}$<0.5, r^2<0.05, P>0.50), but when combined with diet type in an ANCOVA, both frass N and diet type had significant effects at 7 days (Table 2). Diet type also affected the amount of C released from the frass over the 28-day period (Table 2). In both time intervals, less C was released from the meridic diets than from either grass or forb

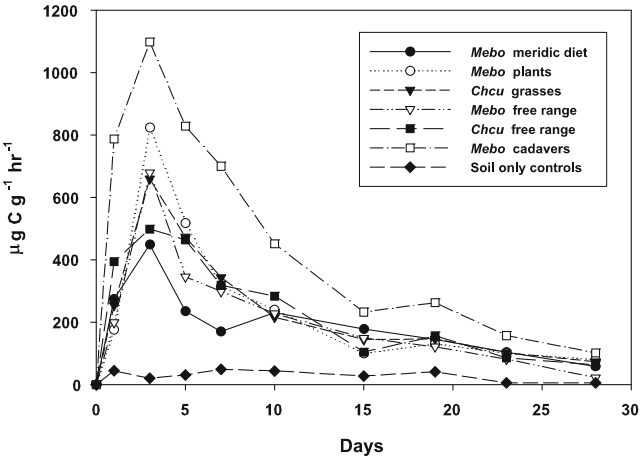

Fig. 1 Rates of C mineralization from soil amended with grasshopper frass during 4 weeks of incubation at 15°C

foods (Fig. 2). There was no effect of grasshopper species on C mineralization after either 7 or 28 days (ANCOVA, $F<1.6$; $P>0.25$). All cadaver treatments resulted in the highest amount of mineralized C (Fig. 1) compared to all other respiration treatments.

Discussion

The N concentration of frass was proportional to the N concentration of the grasshoppers' diet. Nitrogen in frass may come from a combination of several possible sources, including waste products, such as uric acid, remnants of peritrophic membrane, and undigested protein or other nitrogenous compounds. We did not measure absolute amounts of N in diets or frass and so cannot determine whether assimilation rates were similar among diets, but differences among diet types in terms of diet/frass N concentrations were noted with *M. borealis*. Frass derived from

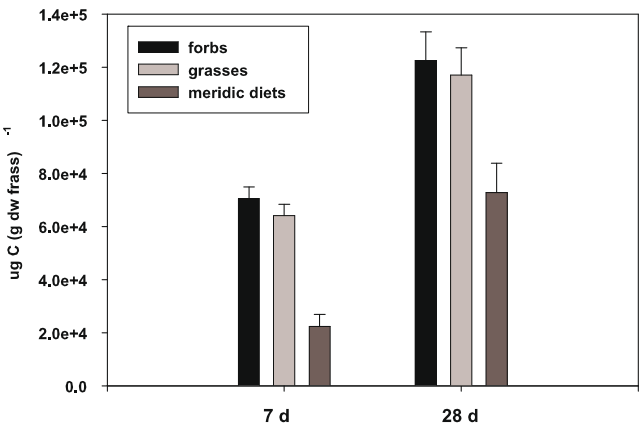

Fig. 2 Cumulative amount of C released per gram of frass from grasshoppers fed with forbs, grasses, or meridic diets. Samples were incubated in soil at 15°C for 7 and 28 days

forbs (dandelion and hawksbeard) had a lower concentration of N than frass derived from grasses or meridic diets for a given level of N in the food. This may indicate greater assimilation of N from forbs. Tougher cell walls in the grasses may have impeded digestion of nutrients (Clissold et al. 2004, 2009); however, the meridic diets had similar ratios of food/frass concentration of N as did the grasses, and assimilation of nutrients from meridic diets tends to be high (Zanotto et al. 1993). Our data do not explain the difference in food/frass ratios of N concentration nor the generality of the differences between assimilation of nutrients from grasses compared to forbs. Further research will be necessary to determine how diet influences the relative contribution of different sources of N in frass.

Frass with less than about 2.0 % N resulted in net N immobilization after 4 weeks. The observed fungal growth on the samples suggests that it was microbial immobilization that was responsible for this decrease. The high rates of C mineralization indicate that the C within frass was readily available as an energy source for microbes. The results for our C mineralization experiment were similar to that of Lovett and Ruesink (1995), who also reported increased C mineralization from soil samples treated with frass. In their study, the peak C mineralization rate, which occurred on the second day of incubation, was approximately 35,000 µg C $(\text{g ADW})^{-1}\text{day}^{-1}$. The peak C mineralization rates in our experiments was somewhat lower, approximately 12,000 to 20,000 µg C $(\text{g ADW})^{-1}\text{day}^{-1}$ for frass from grasshoppers feeding on natural foods. Our frass/soil samples were incubated at 15°C, whereas Lovett and Ruesink (1995) incubated their samples at 21°C.

Grasshopper species had little or no effect on frass properties and mineralization rates, aside from differences in diet, with *M. borealis* consuming forbs and grasses and *C. curtipennis* feeding mainly on grasses. Diet quality explained most of the variation in frass properties and subsequent mineralization rates. Hence, ecosystem properties, such as plant community and soil fertility, have to be considered when assessing the role of insect herbivores on nutrient cycling. For example, an extreme contrast can be made between grasshoppers feeding in fertilized crops, such as those commonly occur in small grains across North America and those feeding on rangeland plants with low levels of N. In the former case, grasshopper frass will release a substantial amount of N within the same growing season, whereas in the latter case, N will be at least temporarily immobilized.

The data show that despite their different diets, the grasshopper cadavers contained almost the same amounts of N and C. Grasshoppers and locusts feeding on different quality diets are known to use post-ingestion mechanisms which allow them to maintain nutritional homeostasis (Zanotto et al. 1993; Clissold et al. 2010). So, because of these

homeostatic mechanisms, grasshoppers will often have the same C and N concentrations in their body tissues independent of their diet quality (Simpson and Raubenheimer 1993; Zanotto et al. 1997; Fagan et al. 2002). Nitrogen in bodies of dead grasshoppers was rapidly mineralized and available for uptake by plants. Net import of N by invading grasshoppers (10 % N by DW=0.0125 gN/grasshopper for medium-sized species) could amount to 2.5 kg/ha during heavy infestations (20 m^{-2}), assuming that the grasshoppers die in the field.

In nature, the C/N ratios of foods and frass will be extremely variable and other factors like soil type and soil temperature and moisture would certainly influence mineralization as well. Our data from the free-ranging grasshopper treatments suggest that naturally foraging grasshoppers in Alaska agroecosystems are able to find enough high N food to produce good quality frass. However, perhaps in other regions or other years, food may be low enough in N to produce frass that results in N immobilization (at least in the short term). We tried to keep our incubation experimental conditions similar to local field conditions as we could, with the samples being incubated at 15°C and soil moisture maintained at 90 % of field capacity. Another point worth mentioning is that our study examined only short-term effects (28 days). In the longer term, it would be expected that the N-immobilizing soil microbes would die off, and at this point, possibly more of the N would be made available. In their 120-day incubation experiment, Lovett and Ruesink (1995) noticed that the frass component of the soil + frass samples continued to immobilize N until day 90. However, after this point, they saw a switch from net N immobilization to net N mineralization.

In these experiments, we tested whether diet had a significant influence on the C and N mineralization rates. Both grasshopper frass and cadaver additions increased the C mineralization potential of soil as compared to soil alone (Fig. 1), but it was clear that the cadaver inputs resulted in the largest increases. The results from this study clearly demonstrate that grasshopper frass and cadavers stimulate microbial growth, which can help to conserve part of the large pulse of N that can occur with frass and cadaver deposition. However, our results also show that when grasshopper frass and cadaver inputs contain high enough concentration of N, they can greatly increase the N mineralization potential of the soil, resulting in positive net mineral N. This pulse in mineral N may increase nutrient cycling and increase the growth of plants.

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