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Positive and negative impacts of insect frass quality on soil nitrogen availability and plant growth

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Abstract Frass deposition to soil is an important pathway by which herbivorous

15 insects impact decomposition and soil nutrient availability. However, little is known
about how frass quality influences ecosystem properties. Here, we examined the effects
of frass quality on the decomposition process, soil nitrogen (N) availability, and plant
growth, using frass of *Mamestra brassicae* (L.) that fed on fertilized or unfertilized
Brassica rapa L. var. *perviridis* Bailey. The frass quality was largely dependent on the
20 host plant quality. Frass excreted by larvae that fed on the fertilized plants had higher N
than that of larvae that fed on the unfertilized plants. The decomposition rate of the frass
did not differ between N-rich and N-poor frass, except during the early decomposition
period. The inorganic N concentration decreased during decomposition in both frass
types. However, difference in the initial inorganic N concentration led to different
25 consequences regarding soil N availability. Furthermore, addition of frass to the soil
differently influenced the growth of *B. rapa* plants depending on the frass quality: plant
biomass was increased by N-rich frass addition but decreased by N-poor frass addition,
compared to the biomass without frass addition. These results indicate that frass quality
is an important factor in determining the impact of herbivorous insects on nutrient

30 dynamics, and that frass positively or negatively influences soil N availability and plant growth, depending on its quality.

Keywords Aboveground-belowground interaction · Decomposition · Fertilization ·

Insect-plant interaction

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Introduction

There is a growing body of evidence that the consumption of living foliage by

herbivorous insects has significant impacts on ecosystem processes, such as

40 productivity and decomposition (Belovsky and Slade 2000; Schowalter 2000; Hunter

2001; Weisser and Siemann 2004). For example, insect herbivory can change litter

chemistry through selective feeding and herbivory-induced responses, which results in

an altered litter decomposition rate (Belovsky and Slade 2000; Schweitzer et al. 2005;

Chapman 2006; Kay et al. 2008). Deposition of insect excrement (i.e., frass and

45 honeydew) can also affect the decomposition process and nutrient dynamics in soil

(Weisser and Siemann 2004). Frass of herbivorous insects contains more labile carbon

(C) than does leaf litter (Lovett et al. 2002). It can stimulate microbial growth (Frost and

Hunter 2004), which in turn increases soil respiration (Lovett and Ruesink 1995),

decomposition rate (Zimmer and Topp 2002), and nitrogen (N) mineralization or

50 immobilization (Lovett and Ruesink 1995, Frost and Hunter 2007, 2008). These impacts

are dependent on the amount of insect frass deposited to soil. In other words, this is a

function of insect population dynamics (Hunter 2001). Therefore, determining the relationship between insect population dynamics and ecosystem processes is a critical issue for understanding the roles of insect herbivores in an ecosystem context (Hunter 2001; Lovett et al. 2002).

On the other hand, effects of frass quality on decomposition process have been paid less attention than effects of frass quantity, despite the fact that the quality of herbivorous insect frass differs depending on host plant quality and insect species identity (Madritch et al. 2007; Kagata and Ohgushi 2011). It is well known that the process of decomposition of plant litter is strongly affected by litter chemicals (Enríquez et al. 1993). In general, litter with high N and phosphorus is decomposed rapidly, whereas litter with high polyphenols and lignin is decomposed slowly (Enríquez et al. 1993; Schädler et al. 2003; Kurokawa and Nakashizuka 2008). Therefore, the quality of insect frass is expected to affect the decomposition process and soil nutrient availability (Madritch et al. 2007).

We previously examined the relationship between the frass quality of cabbage armyworm, *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae), and the leaf quality of

the host plant, *Brassica rapa* L. var. *perviridis* Bailey (Brassicaceae), at various fertilization levels (Kagata and Ohgushi 2011). We showed that the frass quality of *M. brassicae* larvae was strongly affected by the quality of host plant leaves, and that the larval frass had high levels of total N, nitrate-N (NO_3^- -N), and ammonium-N (NH_4^+ -N) when fed on N-rich plant leaves under fertilization.

In the present study, we examined how the frass quality of a herbivorous insect affects the decomposition process, soil N availability and plant growth, using frass of *M. brassicae* larvae fed on *B. rapa* with or without fertilization. Two types of experiments were conducted. One was a frass incubation experiment in a laboratory microcosm to characterize the process of decomposition of frass, by monitoring the frass mass and concentrations of NO_3^- -N, and NH_4^+ -N for five weeks. The other experiment was frass addition to potted *B. rapa* plants to determine the plant growth response to insect frass deposited on the soil surface.

Materials and methods

Collection of insect frass

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One hundred ninety-two *B. rapa* plants (Rakuten, Takii Syubyo Co. Ltd., Kyoto, Japan)

were individually grown in 500-ml pots using nutrient-rich compost (Tanemaki-baido,

Takii Syubyo Co. Ltd., Kyoto, Japan) as the growth medium in a glass-shield

greenhouse at 25°C under natural light conditions. After seeding, plants were watered

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daily. As a fertilized treatment, 96 randomly selected *B. rapa* plants were fertilized with

liquid fertilizer (HYPONeX; N:P:K = 6:10:5, HYPONeX JAPAN Co. Ltd., Osaka,

Japan) which was diluted 30-fold with water. This fertilization level was excessive

beyond the level of inorganic N that *B. rapa* is able to assimilate, but it was within the

range of fertilization used in *B. rapa* culture in Japan (Kagata and Ohgushi 2011). The

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remaining 96 plants were assigned to an unfertilized treatment. Two weeks after seeding,

when the plants reached a four-true-leaf stage, either 50 ml of fertilizer solution or 50

ml of water were supplied to individual pots at one-week intervals. Plants that had

grown for four weeks were used as food for *M. brassicae* larvae to obtain frass.

Eggs of *M. brassicae* were obtained from a laboratory population of the Center for

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Ecological Research, Kyoto University. Egg clusters were placed individually in petri

dishes (9 cm in diameter) in an environmental chamber at 25°C with a 16L8D light cycle. Hatched larvae were reared together until third instar, and thereafter ten larvae were reared per petri dish. The larvae were provided with artificial diet (Insecta LFS, Nihon Nosan Kogyo Co. Ltd., Yokohama, Japan) prior to the frass collection. When the

105 larvae reached sixth (last) instar, approximately 50 individuals were randomly transferred to a rearing container (12 × 27 × 9 cm) and were kept for 12 h without diet to allow them to excrete frass of artificial diet origin. Thereafter, the larvae were provided with mature leaves collected from the potted *B. rapa* described above.

Brassica rapa leaves were replaced with new ones every day and larval frass was

110 collected daily until pupation. The collected frass was stored in a -20°C freezer. Twelve replicates were conducted for each treatment, i.e., frass was collected from approximately 600 larvae for each treatment. A subsample of the frass (about 100 mg) from each rearing container was used for chemical analyses (total C, N, NO₃⁻-N, and NH₄⁺-N). The rest of the frass was pooled for each treatment, and oven dried at 60°C for

115 72 h. They were kept in a -20°C freezer until use for the experiments (see below).

During frass collection, mature leaves of *B. rapa* were also collected for chemical

analyses (total C, N, NO_3^- -N, and NH_4^+ -N) from 12 randomly selected plants for each treatment. They were oven dried at 60°C for 72 h and stored in a freezer until the chemical analyses.

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Frass incubation

The decomposition process of the frass was investigated by incubating frass in an environmental chamber. Six replicates were conducted for each of the following three treatments for each of the six incubation periods (treatments: soil + frass from fertilized plants, soil + frass from unfertilized plants, and soil alone; incubation periods: 0, 1, 2, 3, 4, and 5 weeks, for a total of 108 samples). Soil was the same as used for *B. rapa* culture described above. The soil for the experiment was air-dried for two weeks and passed through a 2 mm sieve before the experiment. *Mamestra brassicae* frass was roughly ground because individual frass pellets had cohered with each other during drying. We placed 5.0 g of the soil in a petri dish (9 cm in diameter), and 0.5 g of frass (approximately corresponding to 25% of the herbivory for one-month-grown *B. rapa*, H. Kagata, unpublished data) was scattered on the soil surface together with 20 ml of

distilled water. The petri dishes were covered and incubated in the dark at 25°C for each
135 incubation period. We did not add water during the incubation period, but soil surface
was kept wet through the period. After incubation, the samples were oven dried at 60°C
for 72 h. After the dry weight was measured, they were stored in a -20°C freezer until
chemical analyses (NO_3^- -N and NH_4^+ -N).

140 Plant growth responses to frass deposition

The effects of insect frass on soil nutrient availability were examined by monitoring the
growth of potted *B. rapa* in response to frass addition in a greenhouse. *Brassica rapa*
plants were cultured as described above, but were not fertilized. The soil mass per pot
145 was approximately 110 g in dry weight. Two sets of experiments in relation to the
amounts of frass were conducted separately. One set was conducted by addition of 0.5 g
of frass to individual pots and the other was done by addition of 2.0 g of frass. These
amounts of frass roughly corresponded to 25% and 100% of the herbivory for
one-month-grown *B. rapa*, respectively (H. Kagata, unpublished data). Each

150 experimental set had three treatments, i.e., addition of frass from fertilized plants,

addition of frass from unfertilized plants, and no frass addition. The potted plants were randomly placed in a greenhouse and rearranged every week. Two weeks after seeding, frass was scattered on the soil surface of the potted *B. rapa* in the frass addition treatments. Eighteen replicates were conducted for each treatment of each experimental set. To measure leaf total N, 3rd, 4th, 5th, 6th, and 7th true-leaves were collected 1, 2, 3, 4, and 5 weeks after frass addition, respectively. The leaves collected were oven dried at 60°C for 72 h, and the dry weight was measured. They were stored in a -20°C freezer until chemical analyses. The aboveground parts of the potted plants were collected six weeks after frass addition. They were oven dried at 60°C for 72 h and weighed. The aboveground biomass of individual plants was determined as the sum of the dry weight of the leaves collected for measuring leaf N and the aboveground part harvested at the end of the experiment.

Chemical analyses

Before chemical analyses, all samples were dried at 60°C for 72 h and ground to fine powder. Total C and N were determined using an elemental analyzer (JM 1000CN,

J-Science Co.,Ltd., Kyoto, Japan). NO_3^- -N and NH_4^+ -N were extracted using 1.5 mol/l

KCl and determined using a continuous flow analyzer (Integral Futura, Alliance

170 Instruments, Frépillon, France).

Statistical analysis

Chemicals of plant leaves and insect frass, and plant biomass were compared among the

175 treatments by one-way ANOVA with the Tukey-Kramer HSD test as a post-hoc test if

necessary. In the frass incubation experiment, differences in mass reduction and

inorganic N concentration were tested by two-way ANOVA (factors: treatment and

time). Post-hoc tests (Tukey-Kramer HSD) were conducted separately for each factor,

i.e., comparisons among the treatments within each time period and comparisons among

180 time periods within each treatment. For leaf N of the plant growth experiment, the

statistical comparison among time periods was not done because the leaf position

differed among time periods. In addition, comparison between 0.5 g and 2.0 g frass

addition was not done because these experiments were conducted separately in different

seasons; the experiments with 0.5 g and 2.0 g frass addition were conducted in January

185 and September, respectively. Percentage data were arcsine-square root transformed prior
to analysis. All analyses were conducted using JMP version 6 (SAS Institute Japan,
Tokyo, Japan).

Results

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Quality of *B. rapa* leaves and *M. brassicae* frass

195 Fertilization significantly altered *B. rapa* leaf chemicals (Table 1). Total C concentration
was slightly increased by fertilization. Total N, NO_3^- -N, and NH_4^+ -N concentrations
were increased by fertilization, although NH_4^+ -N concentration stayed at a low level, i.e.,
less than 0.1% .

Frass chemicals were markedly affected by the quality of *B. rapa* leaves
(Table 1). Carbon concentration in the fertilized treatment was significantly lower than
in the unfertilized treatment. On the other hand, frass N, NO_3^- -N, and NH_4^+ -N
200 concentrations were significantly higher in the fertilized treatment than in the
unfertilized treatment.

Frass decomposition process

205 Both treatment and incubation time had significant effects on the reduction of the

substrate mass, i.e., total mass of frass and soil (Table 2). While marked mass reduction

was not detected in the soil alone treatment throughout the experiment, the soil + frass

treatments lost 170-200 mg of substrate mass in the first week of the incubation (Fig. 1).

This roughly corresponds to decomposition of 35-40% of initial frass mass, assuming

210 that the mass reduction of substrate resulted from frass decomposition. The mass

reduction during the first week in the treatment of soil + frass from fertilized plants was

significantly greater than that in the treatment of soil + frass from unfertilized plants,

but the difference between these two treatments was not statistically significant after

two weeks of incubation (Fig. 1). At the end of the experiment, both of the soil + frass

215 treatments lost approximately 300 mg of substrate mass, compared to the initial mass.

Both treatment and incubation time had significant effects on the reduction of

 NH_4^+ -N and NO_3^- -N concentration (Table 2). Compared to the initial level (week 0),

 NH_4^+ -N significantly decreased in both the soil + frass from fertilized and unfertilized

treatments in the first week of incubation (Tukey-Kramer HSD, $P < 0.05$). Consequently,

220 NH_4^+ -N was still higher in the soil + frass from fertilized plants, but lower in the soil +

frass from unfertilized plants, than in the soil alone treatment (Fig. 2a). NO_3^- -N

decreased in the first week of incubation in both the soil + frass treatments

(Tukey-Kramer HSD, $P < 0.05$), and there was no significant difference between these

two soil + frass treatments (Fig. 2b). NO_3^- -N in the soil alone treatment did not change

225 significantly throughout the experimental period (Tukey-Kramer HSD, $P > 0.05$).

Plant responses to frass deposition

When 0.5 g of frass was added to the potted *B. rapa*, leaf N was significantly higher in

230 *B. rapa* with the frass from fertilized plants than in *B. rapa* without frass two weeks

after the frass addition (Fig. 3a). However, leaf N in *B. rapa* with the frass from

unfertilized plants did not differ significantly from that in *B. rapa* without frass

throughout the experimental period. When 2.0 g of frass was added, leaf N was higher

in *B. rapa* with the frass from fertilized plants than in *B. rapa* without frass one week

235 after the frass addition (Fig. 3b). It was also higher in *B. rapa* with the frass from

unfertilized plants than in *B. rapa* without frass four weeks after the frass addition (Fig. 3b).

Frass quantity and quality influenced the aboveground biomass of *B. rapa*.

When 0.5 g of frass was added, there was no significant difference in the aboveground
240 biomass between the frass from fertilized plants treatment and the no frass treatment
(control) (Fig. 4a). On the other hand, the aboveground biomass decreased in the frass
from unfertilized plants treatment, compared to the control (Fig. 4a). For 2.0 g of frass
addition, the aboveground biomass increased in the frass from fertilized plants treatment
compared to the control (Fig. 4b). In contrast, the aboveground biomass in the frass
245 from unfertilized plants treatment decreased compared to the control (Fig. 4b).

Discussion

Insect frass decomposition process

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Insect frass is known to be rapidly decomposed in an early decomposition process

(Lovett and Ruesink 1995; Madritch et al. 2007). For example, the C mineralization rate

of gypsy moth frass was greatest in the first 10 days during 120 days of incubation (Lovett and Ruesink 1995), and was greater than that of plant litter (Madritch et al.

255 2007). This is because insect frass contains more labile C, which can stimulate the activity of microbial decomposers, than leaf litter (Lovett and Ruesink 1995). We showed that the frass of *M. brassicae* was rapidly decomposed in the first 7 days of incubation (see Fig. 1). The amount of frass decomposed during this period corresponded to 35-40% of the initial frass mass, assuming that the mass reduction of
260 substrate resulted from frass decomposition. In this period, N-rich frass (i.e., frass excreted by the larvae fed on fertilized plants) was decomposed more efficiency than N-poor frass (i.e., frass excreted by the larvae fed on unfertilized plants). However, this difference disappeared after the second week.

In general, decomposition rate is positively correlated with N concentration
265 and negatively correlated with C:N ratio of substrates (Enríquez et al. 1993). This pattern was the case for the first 7 days decomposition of frass in the present study. Why did the pattern disappear after the second week? One possible explanation is that the amount of available C may have limited the activity of microbial decomposers in the

later period of frass decomposition. Although C is one of the essential resources for
270 microbial decomposers, the amount of C in the substrates does not strongly limit the
microbial activity compared to N when the microbes utilize substrates with high C:N
ratio (more than about 30), such as leaf litter (Enríquez et al. 1993; Kaye and Hart 1997).

However, the frass in the present study had extremely low C:N ratio (3.3 for N-rich
frass and 10.7 for N-poor frass), and the two types of frass had similar C concentration.

275 Therefore, the available C in the frass may have been rapidly consumed by the
microbial decomposers during the initial decomposition period, and thereafter the
microbial activity may have been limited by C rather than N in the frass. This may be
one of the reasons that decomposition weight loss was similar between N-rich and
N-poor frass after the second week in the incubation.

280 Thus, the decomposition rate in the two frass treatment did not differ except
during the first week. In addition, changes in inorganic N concentration during frass
decomposition also showed a similar pattern between the two frass treatments: the
concentration of both NH_4^+ -N and NO_3^- -N decreased from the initial concentration
(week 0) during the first 7 days. Such a decrease in inorganic N was also observed in

285 the decomposition of gypsy moth frass (Lovett and Ruesink 1995). These decreases
were likely due to microbial immobilization, denitrification, and ammonia volatilization
(Lovett and Ruesink 1995). Although it is unclear which process worked in the present
study, the stimulation of the microbial activity by the insect frass would have played a
key role in the dynamics of inorganic N, because inorganic N showed little change in
290 the soil alone treatment. Lovett and Ruesink (1995) also suggested that microbial
immobilization is responsible for most of the decrease in inorganic N during the
decomposition of gypsy moth frass. In addition, the $\text{NH}_4\text{-N}$ level in the present study
may be underestimated due to ammonia volatilization because $\text{NH}_4\text{-N}$ analysis was
done using dry sample. It is known that ammonia is volatile (e.g., Kuzhivelil and
295 Mohamed 1987). Hence, ammonia volatilization may be also the reason causing the
decrease in $\text{NH}_4\text{-N}$ level.

On the other hand, the decrease in inorganic N during frass decomposition led
to different outcomes of soil available N, depending on the initial concentration of
inorganic N in the frass: $\text{NH}_4^+\text{-N}$ was increased by adding N-rich frass to the soil
300 whereas it was decreased by adding N-poor frass, compared to the soil alone treatment.

This may be explained by NH_4^+ -N consumption by soil microbes, i.e., N immobilization.

In the addition of N-rich frass, soil microbes would consume NH_4^+ -N derived from the frass but could not deplete it. In contrast, when the N-poor frass was added, soil

microbes would consume not only NH_4^+ -N from the frass but also NH_4^+ -N that was

305 originally present in the soil. Regarding the total inorganic N (NH_4^+ -N and NO_3^- -N),

addition of N-poor frass lowered the inorganic N in the soil, compared to the soil alone

treatment. These results indicate that the insect frass quality strongly influenced soil N

availability, although the overall decomposition rate was little affected by frass quality.

310 Plant growth responses to frass addition

The changes in soil N availability caused by the input of insect frass should influence

plant productivity (Wardle and Bardgett 2004). The present study clearly showed that

addition of *M. brassicae* frass to the soil affected aboveground biomass and leaf N

315 concentration of *B. rapa* plants, and that the impacts were variable depending on frass

quality and quantity.

It is well known that plants and soil microbes compete for N in soil (Kaye and

Hart 1997; Bardgett et al. 2003; Måansson et al. 2009). Måansson et al. (2009) showed that soil microbes were superior in competition to plants for inorganic N in conditions of abundant available C. Consequently, soil inorganic N was immobilized as organic N in microbial tissue, and plant uptake of inorganic N was reduced. In the present study, the aboveground biomass of potted *B. rapa* plants decreased when N-poor frass was added to the soil. This may have been due to the decrease in plant uptake of inorganic N, probably due to microbial N immobilization. This explanation is in accord with our results from the frass incubation experiment showing that incubation of soil + N-poor frass decreased the inorganic N concentration relative to the soil alone treatment. Thus, deposition of insect frass to the soil negatively influenced plant growth during a short-term period in the N-poor frass. In addition, the frass may have some inhibitory effects on plant growth due to plant-derived allelopathic chemicals (Silander et al. 1983). For example, frass of eucalypt-feeding beetle suppressed germination and growth of several herbs because the frass contained eucalypt-derived allelopathic chemicals (Silander et al. 1983). It is known that Brassicaceae plants contain glucosinolates that potentially suppress plant growth (Haramoto and Gallandt 2004). Further chemical

analyses of *M. brassicae* frass would help to test this possibility.

335 In contrast to the N-poor frass, addition of N-rich frass increased the

aboveground biomass of *B. rapa* compared to the no frass treatment, and the outcome

was dependent on the amount of frass. The plant biomass increased when 2.0 g of frass

was added, but did not change when 0.5 g of frass was added, indicating that there is a

threshold in insect frass loadings that affect plant growth. In addition, leaf N increased

340 one or two weeks after N-rich frass addition. This finding suggests that the frass N

rapidly permeates the soil, and plants can utilize the N derived from the frass.

Christenson et al. (2002) and Frost and Hunter (2007) directly demonstrated plant

uptake of the N derived from insect frass, using ¹⁵N-labeled frass in a gypsy moth-oak

system. Such N absorption by plants is likely to be as an inorganic form. In addition,

345 soluble organic N in the frass may also contribute to the N absorption by plants. This is

because insect frass contains much more organic N than inorganic N (Cochran 1985;

Lovett et al. 2002), and plants can use soluble organic N (Kaye and Hart 1997; Bardgett

et al. 2003). Besides the soil process that we discussed above, more detailed

measurements for root and soil microbe biomass, and soil chemical properties, such as

350 pH, organic and inorganic N, would help to understand the plant growth responses to
insect frass.

Positive and negative impacts of insect frass on soil nutrient availability

355 Several studies have hypothesized that effects of herbivores on soil N availability vary
depending on plant N concentration; herbivores would decrease soil N availability when
plant N concentration is low but increase it when plant N concentration is high (Ritchie
et al. 1998; Bakker et al. 2009), through the input of their waste products to soil (Frost
and Hunter 2004, 2007; Fonte and Schowalter 2005), modification of litter quality due
360 to selective feedings (Belovsky and Slade 2000; Schmitz 2009), and induced responses
of plants following herbivory (Chapman et al. 2003; Schweitzer et al. 2005; Chapman
2006; Kay et al. 2008). Although plant quality is considered to be an important
parameter determining herbivore effects on soil N availability (Ritchie et al. 1998;
Bakker et al. 2009), few studies have examined directly the relationship between plant
365 quality and herbivore impacts on soil nutrient availability (but see Bakker et al. 2009).

Our results support the hypothesis that herbivores decrease soil N availability when

plant N is low but increase it when plant N is high through frass excretion, when all other environmental factors are equal.

Nitrogen in insect frass deposited to soil would be immobilized by soil microbes

370 in an early decomposition process, and subsequently inorganic N would temporally decrease in the soil (Lovett and Ruesink 1995). This soil process would potentially have negative impacts on plant growth, as the present study demonstrated for the N-poor frass. Interestingly, we also found that insect frass positively influenced soil N availability and plant growth when the frass contained higher levels of inorganic N, in 375 particular NH_4^+ -N. The frass with high NH_4^+ -N clearly resulted from feeding on host plants with a high level of N (Kagata and Ohgushi 2011). Thus, the present study demonstrated that insect frass quality can both positively and negatively influence decomposition and nutrient dynamics in soil. Note that our results were derived from short-terms and small-scale experiments with relatively high herbivory pressure, which 380 may not be directly applicable to the real field. For instance, effects of insect frass on decomposition would vary with the intensity of herbivory pressure and the length of decomposition period (Hunter 2001; Lovett et al. 2002), and decomposition processes

are largely influenced by biological, chemical, and physical properties of the soil (Aerts
1997; Gessner et al. 2010). Nevertheless, our study clearly illustrated that frass
385 quality ,as well as quantity, was a potentially important factor determining the impacts
of herbivorous insects on decomposition and soil nutrient dynamics, and therefore we
should pay more attention to the insect frass quality to understand the role of insect
herbivores in an ecosystem context.

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Table 1 Chemical characteristics of *B. rapa* leaves and frass of *M. brassicae* larvae.

475 Means (SE) are presented. All values are dry weight basis.

	Fertilized	Unfertilized	<i>P</i> value
Leaf chemicals (%)			
Carbon	41.93 (0.23)	41.04 (0.22)	0.01
Nitrogen	8.53 (0.09)	3.67 (0.14)	< 0.0001
NO ₃ ⁻ -N	0.61 (0.03)	0.03 (< 0.01)	< 0.0001
NH ₄ ⁺ -N	0.09 (<0.01)	0.01 (< 0.01)	< 0.0001
Frass chemicals (%)			
Carbon	35.07 (0.21)	38.43 (0.16)	< 0.0001
Nitrogen	10.75 (0.09)	3.58 (0.07)	< 0.0001
NO ₃ ⁻ -N	0.46 (0.03)	0.08 (< 0.01)	< 0.0001
NH ₄ ⁺ -N	1.35 (0.01)	0.19 (< 0.01)	< 0.0001

Table 2 Results of two-way ANOVAs for mass reduction, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$

480 concentration in the frass incubation experiment.

	<i>df</i>	<i>F</i> value	<i>P</i> value
Mass reduction			
Treatment	2,90	1823.0	< 0.0001
Period	5,90	317.5	< 0.0001
Treatment * period	10,90	80.7	< 0.0001
$\text{NH}_4^+\text{-N}$ concentration			
Treatment	2,90	262.0	< 0.0001
Period	5,90	35.3	< 0.0001
Treatment * period	10,90	5.9	< 0.0001
$\text{NO}_3^-\text{-N}$ concentration			
Treatment	2,90	2203.9	< 0.0001
Period	5,90	322.4	< 0.0001
Treatment * period	10,90	95.4	< 0.0001

Figure legends

485

Fig. 1 Changes in substrate (soil + frass) mass relative to the initial condition. Means $\pm$ SE are presented. Initial mass (at week 0) was 5500 mg for soil + frass treatments and 5000 mg for soil alone treatment. Different letters indicate significant difference between treatments within each time period (Tukey-Kramer HSD; $P < 0.05$).

490

Fig. 2 (a) NH_4^+ -N, and (b) NO_3^- -N concentration of the substrate. Means $\pm$ SE are presented. Different letters indicate significant difference between treatments within each time period (Tukey-Kramer HSD; $P < 0.05$).

Fig. 3 Foliar nitrogen concentration of *B. rapa* after frass addition. (a) 0.5 g of frass addition and (b) 2.0 g of frass addition. Means $\pm$ SE are presented. Different letters indicate significant difference within each time period (Tukey-Kramer HSD; $P < 0.05$).

495

Fig. 4 *Brassica rapa* aboveground biomass six weeks after frass addition. (a) 0.5 g of frass addition and (b) 2.0 g of frass addition. Means $\pm$ SE are presented. Different

500 letters indicate significant difference (Tukey-Kramer HSD; $P < 0.05$).

Fig. 1

505 Fig. 2

Fig. 3

(a) 0.5g frass

(b) 2.0g frass

Fig. 4