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# Inorganic Nitrogen Derived from Foraging Honey Bees Could Have Adaptive Benefits for the Plants They Visit

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## Abstract

In most terrestrial ecosystems, nitrogen (N) is the most limiting nutrient for plant growth. Honey bees may help alleviate this limitation because their feces (frass) have high concentration of organic nitrogen that may decompose in soil and provide inorganic N to plants. However, information on soil N processes associated with bee frass is not available. The objectives of this work were to 1) estimate the amount of bee frass produced by a honey bee colony and 2) evaluate nitrogen mineralization and ammonia volatilization from bee frass when surface applied or incorporated into soil. Two cage studies were conducted to estimate the amount of frass produced by a 5000-bee colony, and three laboratory studies were carried out in which bee frass, surface-applied or incorporated into soil, was incubated at 25°C for 15 to 45 days. The average rate of bee frass production by a 5,000-bee colony was estimated at 2.27 to 2.69 g N month$^{-1}$. Nitrogen mineralization from bee frass during 30 days released 20% of the organic N when bee frass was surface applied and 34% when frass was incorporated into the soil. Volatilized $NH_3$ corresponded to 1% or less of total N. The potential amount of inorganic N released to the soil by a typical colony of 20,000 bees foraging in an area similar to that of the experimental cages (3.24 m$^2$) was estimated at 0.62 to 0.74 g N m$^{-2}$ month$^{-1}$ which may be significant at a community scale in terms of soil microbial activity and plant growth. Thus, the deposition of available N by foraging bees could have adaptive benefits for the plants they visit, a collateral benefit deriving from the primary activity of pollination.

## Introduction

In most terrestrial ecosystems, nitrogen (N) is the most limiting nutrient for plant growth as evidenced by studies showing that addition of inorganic N increases plant productivity [1]. Mechanisms of N limitation include N fixation by clays, competition by microorganisms, reduced N diffusion due to low soil water content, and reduced N mineralization from organic residues due to low N content, acid pH, or low soil temperature and water content [2]. Insects may help alleviate this N limitation by reintroducing N into soil through their feces (frass). Most terrestrial insects are believed to excrete waste N as uric acid [3,4,5], but some insects such as the cotton stainer (*Dysdercus fasciatus*) excrete waste N as allantoin, which is a byproduct of uric acid decomposition [6]. Ammonium ($NH_4^+$) and urea also occur in frass but in comparatively low amounts [7,5]. Decomposition of insect frass through the activity of heterotrophic microorganisms (bacteria and fungi) may lead to net N mineralization or net N immobilization depending on frass composition. Nitrogen mineralization is the conversion of organic N to $NH_4^+$, whereas N immobilization is the conversion of inorganic N ($NH_4^+$ or $NO_3^-$) to organic N. In a field study, Frost and Hunter [8] saw an increase in soil $NH_4^+$ as a result of decomposition of frass from the eastern tent caterpillar (*Malacosoma americanum*). Similarly, Zaady et al. [9] documented net N mineralization from termite (*Ana-*

*canthothermes ubachi* Navas) feces incubated in the dark at 20 to 25°C. In contrast, Lovett and Ruesink [10], working with gypsy moth frass (*Lymantria dispar*), and Kagata and Ohgushi [11], working with cabbage armyworm frass (*Mamestra brassicae* L.), found that frass decomposition led to N immobilization during 5 to 13 weeks of incubation.

When net N mineralization occurs, $NH_4^+$ is the first inorganic N species produced, which may be subsequently converted to nitrate ($NO_3^-$) through the process of nitrification [12]. The $NH_4^+$ produced through mineralization can also be lost to the atmosphere as ammonia gas ($NH_3$), a process called ammonia volatilization [13]. Both inorganic N species ($NH_4^+$ and $NO_3^-$) are available for plant uptake, but some plants have preference of one N species over the other depending on stage of growth, soil pH, and other environmental factors. In general, plants adapted to low pH and reducing conditions usually take up $NH_4^+$, whereas plants adapted to higher pH and oxidizing conditions take up $NO_3^-$ [14]. Plants such as cereals, corn, sugar beets, and many grasses use either form of N, whereas plants such as tobacco, tomato, and potato, prefer a high $NO_3^-/NH_4^+$ ratio [12].

The frass of honey bees (*Apis mellifera* L.) has been used in studies of microbiological properties [15] and nitrogen composition [16] but no information is available on soil N transformations. Therefore, the objectives of this study were to: 1) estimate the amount of bee frass produced by a honey bee colony, and 2)

evaluate N mineralization and $NH_3$ volatilization from honey bee frass when surface applied or incorporated into soil. To accomplish these objectives, we conducted two field studies and three laboratory studies.

## Materials and Methods

No specific permits were required for the described field studies because they were located in general areas of a research farm owned by the University of Georgia. The location is not privately-owned or protected in any way, and the field studies did not involve endangered or protected species.

### Frass Production

Two cage studies were conducted to estimate the amount of bee frass produced by a honey bee colony. In the first study, two cages (1.8 m ×1.8 m ×1.8 m) were established on May 24, 2010, at the Horticulture Farm of the University of Georgia. The bottom of each cage contained Cecil loamy sand soil (fine, Kaolinitic, thermic Typic Kanhapludults) with bermudagrass (Cynodon dactylon L.) and tall fescue (Lolium arundinacea Schreb.) plants growing on it. One honey bee colony was introduced into each of the cages. Each colony included 500 g of bees (about 5000 individuals), two honey combs, and two brood combs. The bees were supplied with water and pollen patties during the experiment (25 g pure pollen from GloryBee Foods, Eugene, OR, mixed with 25 g sugar powder and 10 g honey). The amount of bee frass being deposited in each cage was estimated on eight separate dates from June 5 through June 27 by placing two sheets of corrugated plastic (0.1 m$^2$) inside each cage for 24 or 48 hours. One sheet was placed in one corner of the cage and one sheet was placed at the center of the cage. The frass collected on the plastic sheets was dried at room temperature (23°C) for 24 hours, weighed, and analyzed as described below. The second study, established on August 21, 2010, was similar to the first except that there were four cages and each cage contained only bermudagrass plants. The amount of bee frass deposited in each cage was estimated on six dates from August 30 through September 21, as described above. The bee frass collected in these studies was used for the incubation studies after it was analyzed as described below.

Frass samples were ground and analyzed for pH, total C, total N [17], total P, and other elements (ICP-OES after acid digestion) (Tables 1 and 2). In addition, 1 g frass was extracted with 40 mL 1 M KCl and the extract was analyzed for $NO_3^-$ [18], $NH_4^+$ [19], urea [20], and uric acid. Uric acid content was determined by extracting 1 g frass with 500 mL 0.12 M sodium acetate in a water bath at 50°C for 2 hours. An aliquot of the extract was filtered directly into a 2-mL target vial for HPLC analysis using a Luer-type syringe with a 0.45-μm polypropylene filter. Analysis was performed on a Hewlett-Packard series 1100 HPLC instrument with degasser and 0.05 M $KH_2PO_4$ as mobile phase (prepared from HPLC-grade salt). Separation was achieved using an ODS-2 Hypersil LC column (250×4.6 mm; 5 μm particle sizes, and a UV/VIS detector (290 nm) was used to quantify the extracted uric acid as it eluted.

### Incubation Studies

Three incubation studies were conducted to evaluate N mineralization and $NH_3$ volatilization when bee frass is applied to soil. Studies 1 and 2 were conducted with surface-applied bee frass, whereas Study 3 was carried out with bee frass incorporated (mixed) into the soil. The soil used was collected from the upper 15 cm of an area mapped as Cecil loamy sand (fine, Kaolinitic, thermic Typic Kanhapludults) in the Georgia Piedmont (USA).

The soil sample was air dried and passed through a 2-mm sieve before use. Soil characteristics included 0.203 g silt g$^{-1}$, 0.037 g clay g$^{-1}$, and 0.760 g sand g$^{-1}$. Additional characteristics are presented in Tables 1 and 2. Soil pH was measured with a combination pH electrode in a 1:1 (soil: deionized water) ratio. To determine the H$^+$ buffering capacity of the soil, 20 g soil was mixed with 20 mL 0.01 M $CaCl_2$, a pH measurement was made, and then 2.7 mL 0.023 M $Ca(OH)_2$ was added and allowed to react for 30 min before a second pH measurement was made. Soil was analyzed for particle size by the pipette method [21]. Total C and N were analyzed by dry combustion [17] and total P and other elements by ICP-OES after acid digestion.

**Study 1.** The experimental units were 50-mL beakers (4 cm ID), each containing 20 g (dry weight equivalent) of soil and receiving one of the following treatments: 1) Soil without bee frass +2 mL deionized (DI) water, 2) Soil +2 mL DI water + bee frass surface-applied (20 mg) following water application, or 3) Soil + bee frass surface-applied (20 mg) prior to water application +2 mL DI water. The amount of DI water added was intended to bring the soil water content to 0.10 g $H_2O$ g$^{-1}$ (approximately field capacity), and the timing of addition of DI water was intended to simulate rain before or after surface deposition of bee frass. The rate of frass application corresponded to 16 g m$^{-2}$ which is the approximate amount of bee frass deposited by a colony of 5,000 bees in one month (see results of frass production below). Each experimental unit was placed into a 4-L glass jar containing 100 mL DI water at the bottom to minimize desiccation and an acid trap with 20 mL 0.25 M $H_2SO_4$ to trap volatilized $NH_3$. Treatments were replicated five times and arranged in a completely randomized design inside an incubator set at 25°C for 15 days. Acid traps were changed every 3 days and analyzed for $NH_4^+$ to determine volatilized $NH_3$. At the end of the study, the soil in each experimental unit was extracted with 160 mL 1 M KCl by shaking in a reciprocating shaker set at 120 oscillations per minute for 30 min. The extracts were analyzed for $NH_4^+$ and $NO_3^-$ as described above.

**Study 2.** The experimental units and treatments were the same as those described in Study 1, but this study was conducted for 45 days at 25°C with destructive extraction of four replicates of each treatment at 15, 30, and 45 days of incubation as described above. In addition to $NH_3$ traps, the 4-L glass jars holding the experimental units also contained a beaker with 20 mL 0.2 M NaOH to trap carbon dioxide ($CO_2$). These $CO_2$ traps were changed every 3 days (as the $NH_3$ traps) and were titrated to pH 7 with HCl after adding excess $BaCl_2$.

**Study 3.** The experimental units were the same as those described in Study 1, but the treatments were as follows: 1) Soil + 20 mL DI water and 2) Soil +20 mL DI water + bee frass (20 mg) incorporated into the soil. Treatments were arranged in a completely randomized design inside an incubator set 25°C, and destructive extraction of four replicates of each treatment was carried out at 15 and 30 days of incubation as described above.

### Statistical Analysis

Cumulative net N mineralized and $NH_3$ volatilized from the bee frass was calculated by subtracting inorganic N and volatilized $NH_3$ in controls without bee frass addition. Data of net N mineralized and $NH_3$ volatilized in the incubation studies were analyzed as a completely randomized design using PROC ANOVA in SAS [22]. Data of cumulative $CO_2$ emission at different times were analyzed as a completely randomized design with repeated measures using PROC MIXED in SAS [22] and compound symmetry as covariance structure. Fisher's protected

**Table 1.** pH, buffering capacity, total C, total N, and inorganic and organic N forms in soil and bee frass.

| Material | pH | H$^+$ Buffering Capacity | Total C | Total N | NH$_4^+$-N | NO$_3^-$-N | Uric Acid-N | Urea-N |
|---|---|---|---|---|---|---|---|---|
| | | mmol H$^+$kg$^{-1}$pH$^{-1}$ | g kg$^{-1}$ | | mg kg$^{-1}$ | | | |
| Soil | 6.3 | 5 | 16.5 | 1.3 | 21 | 16 | ND | ND |
| Frass | 5.7 | ND | 531.5 | 51.5 | 624 | 10 | 11,400 | 202 |

ND = not determined.
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LSD was used to separate treatments means, and effects were considered significant when p<0.05.
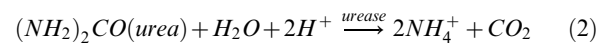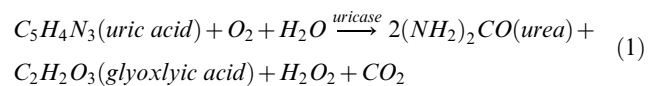
## Results and Discussion

### Frass Production

The average daily amount of frass collected in the first study was $540\pm120$ mg m$^{-2}$ d$^{-1}$ (95% confidence interval) when 5,000 bees foraged in an area of 3.24 m$^2$. Thus, the monthly amount of frass deposited can be estimated at $16.2\pm3.6$ g frass m$^{-2}$ month$^{-1}$, which is equivalent to $0.83\pm0.18$ g N m$^{-2}$ month$^{-1}$ (assuming total N = 51.5 g kg$^{-1}$, Table 1). In the second cage study, the average daily amount of frass was $450\pm170$ mg frass m$^{-2}$ d$^{-1}$ (95% confidence interval). Using similar calculations as for the first study, the amount of frass deposited in the second study can be estimated at $13.5\pm5.1$ g frass m$^{-2}$ month$^{-1}$, which is equivalent to $0.70\pm0.26$ g N m$^{-2}$ month$^{-1}$. Taking into consideration the area of the cages (3.24 m$^2$), the total amount of frass N produced by 5,000 bees in one month can be estimated at $2.69\pm0.58$ g N month$^{-1}$ in the first study and $2.27\pm0.84$ g N month$^{-1}$ in the second study. The significance of these N depositions is discussed below taking into account results of the incubation studies.

The bee frass collected from cages and used in our laboratory studies had a high total N content (51.5 g kg$^{-1}$, Table 1) compared to results by McNally et al. [16] who found values ranging from 23.2 to 40.0 g N kg$^{-1}$. In contrast, our bee frass had a smaller amount of uric acid (22% of total N) than the frass analyzed by McNally et al. (48 to 96% of total N), and the ratio of ammoniacal-N (NH$_4^+$ + NH$_3$) to uric acid-N was low (0.055). In addition, frass pH was 5.7, indicating that the ammoniacal N was mainly present as NH$_4^+$. Nitrate (0.02% of total N) and urea (0.4% of total N) were present in low amounts. These analyses show that 99% of the N in frass was present in organic form. The C:N ratio of the frass was 10.3, which indicated that frass decomposition would be expected to result in net N mineralization. In addition to C and N, bee frass contained significant amounts of P, K, Ca, Mg, and S (Table 2). Thus, frass decomposition may be important in recycling not only N but also other plant nutrients.

### Incubation Studies

**Study 1.** In the first surface-applied study, there was no difference between water-timing treatments (frass added before or after simulated rain) in the percentage of organic N mineralized from bee frass in 15 days (24%, Table 3). This value was similar to the percentage of uric acid-N in the organic N fraction of the frass (22%), which suggests that uric acid may have been the main source of mineralized N. Decomposition of uric acid through the uricase enzyme produces urea, which in turn is hydrolyzed by the urease enzyme to produce NH$_4^+$and CO$_2$ [23]. The hydrolysis of urea consumes H$^+$, which leads to a pH increase, its magnitude depending on initial pH, reaction rate, and H$^+$ buffering capacity of the soil [24].

$$C_5H_4N_3(uric\ acid)+O_2+H_2O \xrightarrow{uricase} 2(NH_2)_2CO(urea)+ \tag{1}$$
$$C_2H_2O_3(glyoxlyic\ acid)+H_2O_2+CO_2$$

$$(NH_2)_2CO(urea)+H_2O+2H^+ \xrightarrow{urease} 2NH_4^+ + CO_2 \tag{2}$$

$$NH_4^+ \leftrightarrow NH_3 + H^+ \tag{3}$$

As pH at the site of urea hydrolysis increases, NH$_4^+$ converts to NH$_3$ which in turn may be evolved from the soil in gaseous form [13]. In this study, the addition of DI water before or after applying frass did not affect the amount of volatilized NH$_3$ (average = 0.8% of the total N applied, Table 3). The low amount of NH$_3$ loss agrees with results from a study with gypsy moth frass in which volatilized NH$_3$ was 0.1% of total N in the frass [25]. The low amount of volatilized NH$_3$ was probably due to the relatively low pH values of the frass (5.7) and soil (6.3) coupled to the H$^+$ buffering capacity of the soil (5 mmol H$^+$ kg$^{-1}$ pH$^{-1}$) and frass. If the entire mineralized N is assumed to have been derived from the conversion of uric acid to urea, which in turn was subsequently hydrolyzed in the upper 2 mm of soil, then using the measured buffering capacity of the soil it is possible to estimate an increase in

**Table 2.** Selected elemental composition of soil and bee frass.

| Material | P | Ca | K | Cu | Mg | S | Fe | Zn | B |
|---|---|---|---|---|---|---|---|---|---|
| | mg kg$^{-1}$ | | | | | | | | |
| Soil | 415 | 669 | 553 | <0.4 | 251 | 238 | 10,203 | 11 | <0.8 |
| Bee Frass | 7,228 | 3,465 | 11,476 | 7 | 1,947 | 2,689 | 794 | 61 | 68 |

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**Table 3.** Nitrogen released (initial inorganic N + mineralized N), N mineralized, and $NH_3$ volatilized from bee frass in two surface-applied studies and one incorporated study held at 25°C for 15 to 45 days.

| Study | Days | Treatment | Nitrogen Released | Nitrogen Mineralized | NH₃ Volatilized |
|---|---|---|---|---|---|
| | | | % of Total N | % of Organic N | % of Total N |
| 1 | 15 | Water+Frass(surface)[‡] | 25.9a[†] | 25.0a | 1.0a |
| 1 | 15 | Frass(surface)+Water | 24.2a | 23.2a | 0.6a |
| | | | | | |
| 2 | 15 | Water+Frass(surface) | −3.1b | −4.3b | 0.1a |
| 2 | 15 | Frass(surface)+Water | 19.3a | 18.3a | 0.1a |
| | | | | | |
| 2 | 30 | Water+Frass(surface) | 19.2a | 18.3a | 0.2a |
| 2 | 30 | Frass(surface)+Water | 22.8a | 22.0a | 0.2a |
| | | | | | |
| 2 | 45 | Water+Frass(surface) | 18.9a | 17.9a | 0.2a |
| 2 | 45 | Frass(surface)+Water | 24.2a | 23.5a | 0.2a |
| | | | | | |
| 3 | 15 & 30 | Frass (Incorporated) | 34.8 | 34.2 | – |

[†]Within a column and study period, means followed by the same letter are not significantly different according to Fisher's protected. LSD at $p<0.05$.
[‡]Water+Frass(surface) =2 mL of water added to 20 g of soil, then 20 mg of frass applied on the surface.
Frass(surface)+Water =20 mg of frass applied on the surface of 20 g of soil, then 2 mL of water added to soil.
Frass (Incorporated) =20 mg frass incorporated into 20 g soil, then 2 mL of water added to soil.
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pH of 0.1 units. Such a small pH increase from 6.3 to 6.4 would lead to minimal $NH_3$ losses as observed in this study.

There was no difference between water-timing treatments in the amount of inorganic N released (initial inorganic N + mineralized N) from bee frass in 15 days (average of 25% of applied N), but $NO_3$-N expressed as percentage of total inorganic N released $((NH_4^+ + NO_3^-)$-N$)$ was 22% in the treatment that received water before bee frass and 65% in the treatment that received water after the frass. These results suggest that bee frass may have contained water-soluble compounds that partially inhibited nitrification. When water was added after bee frass these compounds may have been leached into the soil, thereby eliminating this partial inhibition.

**Study 2.** In the second surface-applied study, there was net N immobilization at 15 days in the treatment that received DI water before applying bee frass (Table 3). This suggests that the bee frass contained labile organic compounds whose decomposition led to N immobilization as has been observed in gypsy moth frass [10]. In contrast, when DI water was added after applying bee frass, 18% of the organic N was mineralized in 15 days. The addition of water after frass caused a fast colonization by fungi that was evident by extensive hyphal development on the soil surface. This fungal development may have been responsible for the observed N mineralization. In addition, water added after the frass may have leached into the soil those compounds that caused N immobilization in the treatment where water was added before the frass.

It is interesting to note that the total $CO_2$ emission was lower in treatments with frass than in the control treatment (Fig 1). This suggests that compounds present in the frass decreased soil respiration. Furthermore, starting on day 13, cumulative $CO_2$ emission was lower in the treatment where DI water was added after the frass when compared to the other treatments (Fig. 1). This effect may have been caused by the added water leaching inhibiting compounds from the frass into the soil.
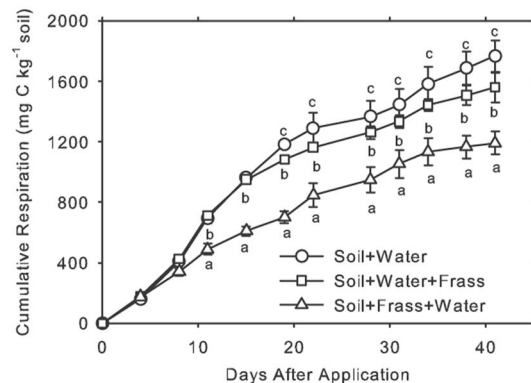


**Figure 1. Cumulative respiration from three treatments in Study 2.** The following treatments were incubated at 25°C for 45 days: 1) Soil+Water = soil (20 g) without bee frass; 2) Soil+Water+Frass = soil (20 g) with simulated rain (2 mL) before surface application of bee frass (20 mg); 3) Soil+Frass+Water = soil (20 g) with simulated rain (2 mL) after surface application of bee frass (20 mg). Bars are standard deviations.
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In spite of the differences observed between treatments on day 15, at 30 and 45 days of incubation both treatments showed net N mineralized averaging 20% of the organic N. As in the first study, this value is similar to the percentage of uric acid in the organic N fraction of the frass. Also, in agreement with results from the first study, the total amount of $NH_3$ volatilized was minimal in both treatments (0.2% of applied N, Table 3). At 30 and 45 days of incubation, there was no difference between water-timing treatments in the amount of inorganic N released from bee frass (average of 21% of applied N, Table 3). In both treatments, more

than 90% of the released inorganic N was in the form of $NO_3^-$, which indicates that conditions were adequate for nitrification.

**Study 3.** In this third study in which bee frass was incorporated into the soil, the amount of organic N mineralized and the amount of inorganic N released from bee frass was the same at 15 and 30 days of incubation (Table 3). However, the amount of $NO_3$-N released expressed as percentage of total inorganic N was 63% at 15 days and 98% at 30 days. These results indicate that nitrification did not proceed as fast as N mineralization. The percentage of organic N mineralized (34%) was greater than the percentage of uric acid in the organic N fraction, which indicates that incorporating bee frass into the soil allowed decomposition of additional N-containing organic compounds. These compounds may have been products of uric acid decomposition such as allantoin and amino acids [6].

In general, our results of net N mineralization seem to agree with results by Frost and Hunter [8] who saw an increase in $NH_4^+$ in soil as a result of decomposition of eastern tent caterpillar (*Malacosoma americanum*) frass. In contrast, our general results differ from other studies that have found net N immobilization. For example, Kagata and Ohgushi [11] observed net N immobilization when incubating cabagge armyworm (*Mamestra brassicae L.*) frass (C:N = 3.2 to 4.9) with soil for five weeks. Similarly, Lovett and Ruesink [10] incubated gypsy moth (*Lymantria dispar*) frass (C:N = 20) with soil for 120 days and observed N immobilization during the first 90 days, with most of the N immobilized during the first 10 days. Our bee frass had a C:N ratio of 10 (intermediate between the studies referenced above) and led to net N mineralization. Thus, as is the case for crop residues [26], C:N ratio by itself may not be sufficient to determine whether a given frass will mineralize or immobilize N. Our results suggest that uric acid content in bee frass may be a good indicator of mineralizable N when bee frass is surface applied. Gordillo and Cabrera [27] found that uric acid is also a good indicator of mineralizable N in poultry litter. Thus, using bee frass composition to estimate mineralizable N may be a useful approach because the composition of bee frass varies with bee diet [16]. Additional studies should be conducted with different bee frass samples to determine if uric acid content could be used to estimate mineralizable N.

### Significance of Inorganic N released

The total amount of frass N (organic + inorganic N) deposited by 5,000 bees in one month was estimated at 2.27 to 2.69 g N month$^{-1}$. Assuming that the average inorganic N released from bee frass is similar to the average value observed in the incubation studies (22% of total N), the amount of inorganic N released from the bee frass of 5,000 bees in one month would be 0.50 to 0.59 g inorganic N month$^{-1}$. This amount of N was derived from colonies with about 5,000 bees, but an average bee colony may have 20,000 bees in spring [28] and therefore may result in a monthly release of inorganic N of about 2.0 to 2.4 g inorganic N month$^{-1}$. Foragers can fly several kilometers from the nest, but the most common distance is 600–800 m [29]. More significantly, owing to the celebrated group recruitment behavior of honey bees [30], the concentration of foragers can for a period of days be local and intense – even to the level of patches or individual plants (ie., a scale congruent to the cages used in our study). The area and amount of time in which such concentrated bee foraging occurs is a product of diverse factors including bloom phenology, bloom density, pollinator populations, and weather, but if it is assumed that concentrated foraging by 20,000 bees occurs during one month in an area similar to that of the cages (3.24 m$^2$), this would result in a monthly release of inorganic N of 0.62 to 0.74 g N m$^{-2}$ month$^{-1}$ which can be expected to exceed the N deposition otherwise realized from non-concentrated background deposition of insects or other fauna.

Inorganic N release at this scale may have system-level impacts in terms of soil microbial activity and plant growth for the plant communities within the foraging range of a honey bee colony. The estimated amount of inorganic N released (0.62 to 0.74 g inorganic N m$^{-2}$ month$^{-1}$) is within the range of inorganic N mineralized from soil organic matter in Coastal Plain soils. For example, Egerkraut et al. [31] found that the amount of N mineralized from the upper 120 cm of a Norfolk soil in southern Georgia (USA) ranged from 2.6 to 6.7 g inorganic N m$^2$ in 4 months, which corresponds to 0.65 to 1.7 g inorganic N m$^2$ per month. Thus, concentrated bee foraging could provide an amount of inorganic N similar to that released from soil organic matter in similar Coastal Plain soils, and therefore may have ecological significance. It should be noted, however, that where concentrated bee foraging does not occur, the amount of inorganic N released from deposited bee frass is not likely to be significant for plant growth.

Our results raise the fascinating question whether this ephemeral, albeit intense deposition of available N could have adaptive benefits for the plants visited by bees, a collateral benefit deriving from the primary activity of pollination. A similar collateral benefit was noted when the flight activity of honey bees was shown to interrupt feeding by herbivorous caterpillars [32]. These kinds of indirect interactions between members of food webs warrant more attention to elucidate their evolutionary and community-regulating significance.

### Conclusions

The bee frass used in our studies had about 99% of total N in organic form with 22% of the organic N as uric acid and 0.4% as urea. Mineralization of bee frass N during 30 days at 25°C released 20% of the organic N as inorganic N when bee frass was surface applied and 34% when frass was incorporated into the soil. Our results suggest that the main source of mineralizable N was the uric acid present in the frass. Ammonia volatilization losses corresponded to 1% or less of total N. To our knowledge, this is the first study that evaluated N mineralization and $NH_3$ volatilization from bee frass. The potential amount of inorganic N released from a typical colony of 20,000 bees foraging in a small area (3.24 m$^2$) was estimated at 0.62 to 0.74 g N inorganic m$^{-2}$ month$^{-1}$, which may be significant at a community scale in terms of soil microbial activity and plant growth. Thus, the deposition of plant-available N by foraging bees could have adaptive benefits for the plants they visit, a collateral benefit deriving from the primary activity of pollination. This is an example of many kinds of indirect interactions between members of food webs that may have important but poorly understood regulating effects on ecological communities.

### Acknowledgments

### Author Contributions

# References

1. Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochem 13:87–115.
2. Kaye JP, Hart SC (1997) Competition for nitrogen between plants and soil microorganisms. Trends Ecol Evol 12:139–143.
3. Wigglesworth VB (1950) The principles of insect physiology, 4th ed. London:Methuen. p. 364.
4. Bursell E (1965) Nitrogenous waste products of the tsetse fly, *Glossina morsitans*. J Insect Phys 11: 993–1001.
5. Lovett GM, Christenson LM, Groffman PM, Jones CG, Hart JE, et al. (2002) Insect defoliation and nitrogen cycling in forests. Bioscience, 52: 335–341.
6. Berridge MJ (1965) The physiology of excretion in the cotton stainer, *Disdercus fasciaturs* Signoret. III. Nitrogen excretion and excretory metabolism. J Exp Bio 43: 535–552.
7. Wigglesworth VB (1972) The Principles of insect Physiology, 7th edn. Chapman 8 Hall, London.
8. Frost CJ, Hunter MD (2004) Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. Ecology 85:3335–3347.
9. Zaady E, Groffman PM, Shachak M, Wilby A (2003) Consumption and release of nitrogen by the harvester termite *Anacanthothermes ubachi* Nava in the northern Negev desert, Israel. Soil Biol Biochem 35: 1299–1303.
10. Lovett GM, Ruesink AE (1995) Carbon and nitrogen mineralization from decomposing gypsy moth frass. Oecologia, 104: 133–138.
11. Kagata H, Ohgushi T (2012). Positive and negative impacts of insect frass quality on soil nitrogen availability and plant growth. Popul. Ecol. 54:75–82.
12. Havlin JL, Beaton JD, Tisdale SL, Nelson WL (2005) Soil Fertility and Fertilizers, 7th edn., Pearson Prentice Hall, New Jersey.
13. Kissel DE, Cabrera ML (2005) Ammonia in soils. In: Encyclopedia of soils in the environment. Elsevier Publishing Co. p. 56–64.
14. Maathuis F (2009) Physiological functions of mineral macronutrients. Curr Opin Plant Biol 12:250.
15. Gilliam M, Prest DB (1987) Microbiology of feces of the larval honey bee, *Apis mellifera*. J. Inverteb Patho 49: 70–75.
16. McNally JB, McCaughey WF, Standifer LN, Todd FE (1965) Partition of excreted nitrogen from honey bees fed various proteins. J Nutrition 85:113–116.
17. Nelson DW, Sommers LW (1982) Total carbon, organic carbon, and organic matter. In: Page Al et al. editors. Methods of soil analysis. Part 2. 2nd. Ed. Agron. Monogr. 9, ASA and SSSA, Madison, WI. p. 539–580.
18. Keeney DR, Nelson DW (1982) Nitrogen – Inorganic forms. In: Page AL et al, editors. Methods of soil analysis. Part 2. 2nd. Ed. Agron. Monogr. 9, ASA and SSSA, Madison, WI. p. 643–698.
19. Crooke WM, Simpson WE (1971) Determination of ammonium in Kjeldahl digest of crops by an automated procedure. J Sci Food Agric 22:9–10.
20. DeManche JM, Curl E Jr, Coughenower DD (1973) An automated analysis of urea in seawater. Limnol Oceanogr 16:686–689.
21. Miller WP, Miller DM (1987) A micro-pipette method for soil mechanical analysis. Commun Soil Sci Plant Anal 18:1–15.
22. SAS Institute Inc. (2008) SAS/STAT 9.2 User's Guide. SAS Inst., Cary, NC.
23. Rothrock MJ, Cook KL, Warren JG, Eiteman MA (2010). Microbial mineralization of organic forms of nitrogen in poultry litters. J Environ Qual 39:1848–1857.
24. Kissel DE, Cabrera ML, Vaio N, Craig JR, Rema JA, et al. (2009) Forest floor composition and ammonia loss from urea in a loblolly pine plantation. Soil Sci Soc Am J 73:630–637.
25. Lovett GM, Hart JE, Christenson LM, Gones CG (1998) Caterpillar guts and ammonia volatilization: Retention of nitrogen by gypsy moth larvae consuming oak foliage. Oecologia, 117: 513–516.
26. Cabrera ML, Kissel DE, Vigil MF (2005) Mineralization from organic residues: Research Opportunities. J Environ Qual 34:75–79.
27. Gordillo RM, Cabrera ML (1997) Mineralizable nitrogen in broiler litter: I. Effect of selected litter characteristics. J Env Qual. 26: 1672–1679.
28. Winston ML (1992) The honey bee colony: Life history, 73–101. In J. M. Graham (ed.), The hive and the honey bee, 4th ed. Dadant & Sons, Hamilton, IL.
29. Visscher PK, Seely TD (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology 63:1790–1801.
30. Frisch von K (1967) The Dance Language and Orientation of Bees. Harvard University Press, Cambridge, MA.
31. Egelkraut TM, Kissel DE, Cabrera ML, Adkins W (2003) Predicting N mineralized in a Georgia Costal Plain. Nutr Cycl Agroecosyst 66:1–12.
32. Tautz J, Rostás M (2008) Honeybee buzz attenuates plant damage by caterpillars. Current Biology 18(24): R1125–R1126.