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Increased Forest Soil CO₂ and N₂O Emissions During Insect Infestation

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Abstract: Forest soils are major sinks of terrestrial carbon, but this function may be threatened by mass outbreak events of forest pests. Here, we measured soil CO₂-C and N₂O-N fluxes from a Scots pine (*Pinus sylvestris* L.) forest that was heavily infested by the nun moth (*Lymantria monacha* L.) and an adjacent noninfested (control) forest site during one year. In the infested forest, net emissions of CO₂-C were higher during main defoliation, summer and autumn, while indications of increased N₂O-N emissions were found at one sampling date. On basis of this, a microcosm incubation experiment with different organic matter treatments was conducted. Soil treatments with needle litter, insect feces plus needle litter, and insect feces showed 3.7-, 10.6-, and 13.5-fold higher CO₂-C emissions while N₂O-N of the insect feces plus needle litter, and insect feces treatment was 8.9-, and 10.4-fold higher compared with soil treatments without added organic matter (control). Hence, the defoliation in combination with high inputs of organic matter during insect outbreaks distinctly accelerate decomposition processes in pine forest soils, which in turn alters forests nutrient cycling and the functioning of forests as carbon sinks.

Keywords: CO₂; N₂O; insect outbreak; frass; litter; soil emissions; nun moth; disturbances; climate change

1. Introduction

The worldwide forest area affected annually by insect outbreaks amounts about 36.5 million hectares [1], thereby representing a thread to the function of forests as a carbon sink [2–5]. Insect defoliation not only leads to decreased canopy biomass, tree growth, and inhibits the production new foliage, but can be accompanied by changes in the N nutritional status of infested trees (e.g., decreased net N uptake, N accumulation in fine roots and needles) [6,7]. Defoliation of forest areas during forest pest outbreaks distinctly increase organic input into the soil in the form of insect feces, cadavers, litter, and other plant material [7–9]. Such changes of litter quality and quantity affect the soil organic matter composition [10], and alter nutrient cycling [11–13]. Depending on the outbreak intensity, insect feces can account for up to 46% of the total litterfall amount in Scots pine forests [14]. The easy soluble structure of insect feces, with high amounts of labile C and extractable N, can facilitate nutrient release in soils [15–17]. Therefore, decomposition processes in the course of defoliations may be enhanced [10,18], thereby triggering CO₂ emissions from forest soils [15,19,20]. In contrast, findings from xylophagous insects (compared to phytophagous insects) showed contradictory results. For example, Ponderosa pine (*Pinus ponderosa* Laws.) stands infested by different bark beetle species (Scolytinae) [21] as well as temperate mixed forests infested by the emerald ash borer (*Agrilus planipennis* F.) [22] showed no effect on soil CO₂ emissions. Bark beetle

induced tree diebacks in lodgepole pine (*Pinus contorta* Dougl. ex Loud.) forests caused even decreased CO₂ emissions [23,24].

N₂O flux changes during insect outbreaks are less well studied. No effects on N₂O emission and nitrate leaching were detected in a manipulation experiment with intensive defoliation of hybrid poplar (*Populus × euroamericana* cv. *Eugenei*) stands by gypsy moth (*Lymantria dispar* L.) [25]. A rather decelerating effect on nutrient cycling in the course of insect outbreaks, with reduced litter decomposition and accumulation of the soil nitrogen storage was assumed by Madritch and Lindroth (2015) [26], Verkaik et al. (2006) [27], and Ritchie et al. (1998) [18], which may be related to high amounts of tannins in feces, that can build recalcitrant protein–tannin complexes, and impair soil microbial activity. Therefore, nitrogen deriving from insect outbreaks is hypothesized to be rather redistributed in the ecosystem than to be lost [28].

Carbon and nitrogen cycling is a function of complex effects and interdependencies between climate, topography, soil properties and microbial soil community structures [25]. Moreover, forest soils seem to have a certain resistance to biotic disturbances. This may explain why effects of organic input from insect outbreaks on microbial respiration or nitrogen immobilization in soils are often only detectable at relatively high levels of defoliation (>70%) [19,29]. Nevertheless, the effects of forests pests on greenhouse gas emissions are not sufficiently understood to predict environmental effects. The lack of knowledge about C and N balances at the soil–atmosphere interface during biotic disturbances contributes to this unpredictability.

In this study, we analyzed CO₂-C and N₂O-N fluxes in the course of a nun moth (*Lymantria monacha* L.) outbreak in Scots pine (*Pinus sylvestris* L.) forests in Germany. Moth larvae regularly hatch in April/May, feed on pine needles until July, and can completely defoliate trees during this period [30]. We quantified net CO₂-C and N₂O-N fluxes from a forest soil in an infested compared with a noninfested forest stand. In a consecutive microcosm incubation experiment, we analyzed net CO₂-C and N₂O-N fluxes under controlled conditions with treatment additions of pine needles, insect feces, and pine needles plus insect feces. We expected that (i) decomposition processes would be accelerated by organic inputs in the course of the insect outbreak and (ii) CO₂-C and N₂O-N emissions from soils would respond to these biogeochemical changes.

2. Materials and Methods

2.1. Field Measurement

The measurements were conducted in a 65-year-old Scots pine forest (52°8′38″ N, 13°45′14″ E, 42 m above sea level) heavily infested by the nun moth (~80% crown defoliation) compared with a noninfested 65-year-old Scots pine forest growing on a site with similar site conditions (52°9′29″ N, 13°36′47″; 35 m above sea level). Both forests are characterized as “white moss pine forests” (*Leucobryo-Pinetum* W.Mat.) grown on podzol (Food and Agriculture Organization of the United Nations (FAO) classification) on aeolian sand (0.2–0.63 mm), with little gravel and a pH (1:10 in H₂O) ranging from 3.2 to 3.9 in the mineral soil (Ah) horizon. Soil C/N ratio of the infested site was 29.4 and of the noninfested site 30.3. Both sites did not differ significantly in their soil microbial community composition in early May prior the nun moth population peak [31]. Average annual temperature was 9.2 and 10.8 °C and average annual precipitation was 611 and 474 mm for the study sites in 2013 and 2014, respectively (German Federal Meteorological Service (DWD) and Climate Data Center (CDC), Weather Station Lindenberg (Station ID: 3015), 13.07.2018, see also Supplementary Figure S1; for a description of site properties, see [6,31]). We measured the CO₂-C and N₂O-N fluxes across one year (August 2013–July 2014), i.e., on three dates in 2013 (August–October with *n* = 18 for each date) and five dates in 2014 (March–July with *n* = 30 for each date). The study sites were arranged in a paired sample comparison of noninfested versus infested forests that were located in spatial proximity to each other with *n* = 9 in 2013 and *n* = 15 in 2014 for each plot.

To quantify soil CO₂-C and N₂O-N emissions, a polyvinyl chloride (PVC) lid (25 cm diameter, 13 cm high) was applied to a cylindrical PVC-U-frame (25 cm diameter, 10 cm high) which was permanently inserted (7–8 cm deep) in the organic layer and Ah horizon of sampling sites. Litter was not removed within the frames. CO₂-C fluxes were measured with a four-point-sampling method, where 20 mL air samples were taken with a syringe via a septum from the closed chamber after 0, 20, 40, and 60 min following its sealing and stored in evacuated glass exetainers. CO₂-C and N₂O-N concentrations were determined by gas chromatography (ECD, Shimadzu, Duisburg, Germany). Fluxes were calculated from the linear change of the gas concentrations during chamber closure, the volume of the chamber and the enclosed surface area, according to Lessard et al. (1993) [32]. Values were corrected for air temperature and air pressure using the following equation:

$$\text{factor} = \frac{\text{air pressure (pa)} \times \text{molar weight } \left(\frac{\text{g}}{\text{mol}}\right)}{\text{gas constant } \left(\frac{\text{J}}{\text{mol K}}\right) \times \text{temperature (K)}} \times 10^{-3} \quad (1)$$

and projected to one square meter and one hour. Simultaneously, temperature of the top 10 cm soil depth, gravimetric soil water content of the Ah, and sampling time were recorded for each plot.

2.2. Incubation Experiment

For the microcosm incubation experiment, randomly collected upper mineral soil (Ah) of the control site was used, homogenized, sieved at 2 mm and 200 g fresh weight were transferred to 20 glass incubators (1000 cm³). Incubators were attached to an automated gas chromatographic system (GC) with a ⁶³Ni electron capture detector (ECD) for the measurement of CO₂ and N₂O concentrations (Shimadzu, Duisburg, Germany) (for a description, see also [33]). The air space of the incubators was flushed with synthetic air and the flux was calculated by determining the differences between inlet air and exhausted air (12 measurements per incubator and day). Experimental runtime was 31 days, including six days of soil pre-incubation. Treatments were added on day seven in form of feces, Scots pine needle litter and a mixture of both with five biological replicates for each treatment. The feces was produced under laboratory conditions from *Dendrolimus pini* L. Feces was mixed, dried at 20 °C for 72 h and needle and bark residues were removed. Needle litter was collected from the noninfested control site in 2014 and dried at 20 °C for 72 h. The total amount of needle and feces input was 5 g dry-weight-equivalent of total C, resulting in the addition of 49.2 mg feces (feces treatment), 48.9 mg needle (needle treatment) and 24 mg of both (feces plus needle litter treatment), respectively, per gram soil. Temperature was constant 20 °C and no light exposure during the experiment. On day 4 and day 18, 60 mL dH₂O were added to the incubators. The amount of added water was adjusted to reach 70–80% of the soils maximum water holding capacity. The analyses of element contents of Aluminum (Al), Calcium (Ca), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), Sulphur (S) of the used soil, feces, and needle litter samples are given in Supplementary Table S1. Values are based on HNO₃ extraction (described in [34]) and subsequent measurement by using an ICP-OES (iCAP 6300 Duo VIEW ICP Spectrometer, Thermo Fischer Scientific GmbH, Dreieich, Germany). For the measurement of total carbon (C_{tot}) and nitrogen (N_{tot}) content soil was dried at 105 °C for 24 h, finely ground, and analyzed by a total organic carbon analyzer multi C/N (Analytik Jena, Jena, Germany).

2.3. Statistical Analyses

Statistical analyses were conducted in R 3.3.3 [35]. All data sets were tested for distribution of normality and homogeneity of variances by applying the Shapiro–Wilk test and Levene’s test, respectively. CO₂-C and N₂O-N fluxes were analyzed separately for each of the seven sampling dates by paired Wilcoxon signed-rank tests, with *n* = 18 (for each of the three the dates in 2013), and *n* = 30 (for each of the five dates in 2014), respectively. The Kruskal–Wallis test was used to detect differences between the accumulated CO₂-C and N₂O-N fluxes from the four treatments of

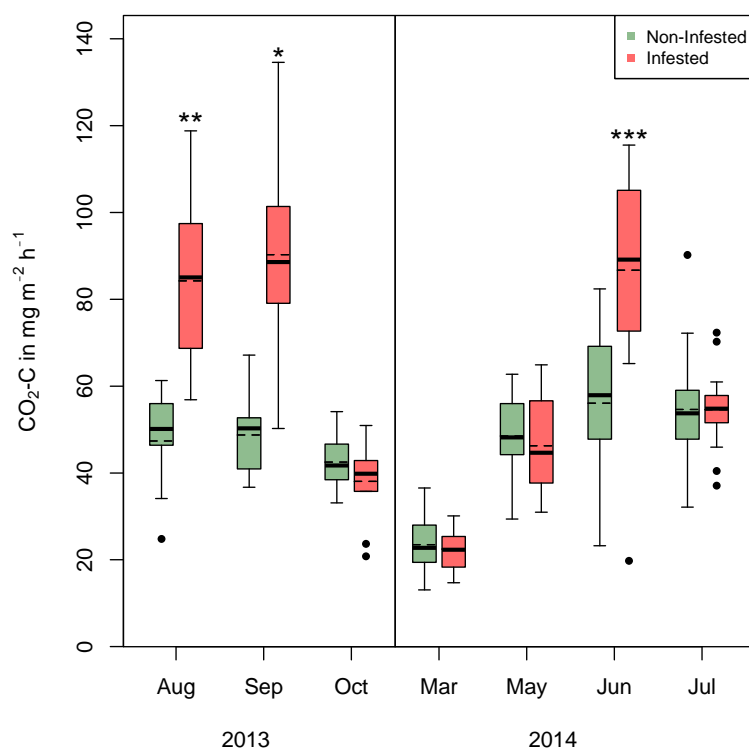
the incubation experiment. In addition, Spearman's rank correlations (r_s) were used to assess the relationships between soil greenhouse gas fluxes ($\text{CO}_2\text{-C}$ and $\text{N}_2\text{O-N}$) and soil temperature as well as soil water content from the field study.

3. Results

3.1. Field Measurement

$\text{CO}_2\text{-C}$ fluxes averaged $47.33 \pm 7.83 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ in noninfested forests and $61.28 \pm 21.98 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ in infested forests. They considerably increased during summer, early in the autumn and during main defoliation in the infested forest site with 1.8- ($p = 0.004$), 1.9- ($p = 0.016$) and 1.5-fold ($p < 0.001$) increased emissions in August and September 2013 and June 2014, respectively (Figure 1). $\text{N}_2\text{O-N}$ fluxes averaged $0.27 \pm 0.41 \text{ } \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ in noninfested forests and $0.91 \pm 0.74 \text{ } \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ in infested forests. $\text{N}_2\text{O-N}$ emissions were significantly increased in the infested forest in October 2013 ($p = 0.039$), while $\text{N}_2\text{O-N}$ flux of the noninfested forest was negative from August 2013 to March 2014 (Figure 2).

$\text{CO}_2\text{-C}$ fluxes correlated positively with soil temperature ($r_s = 0.738$, $p = 0.046$, Supplementary Figure S2), but not with soil water content ($r_s = -0.700$, $p = 0.233$). $\text{N}_2\text{O-N}$ emissions were neither correlated with soil temperature ($r_s = -0.095$, $p = 0.840$) nor with soil water content ($r_s = -0.300$, $p = 0.683$).



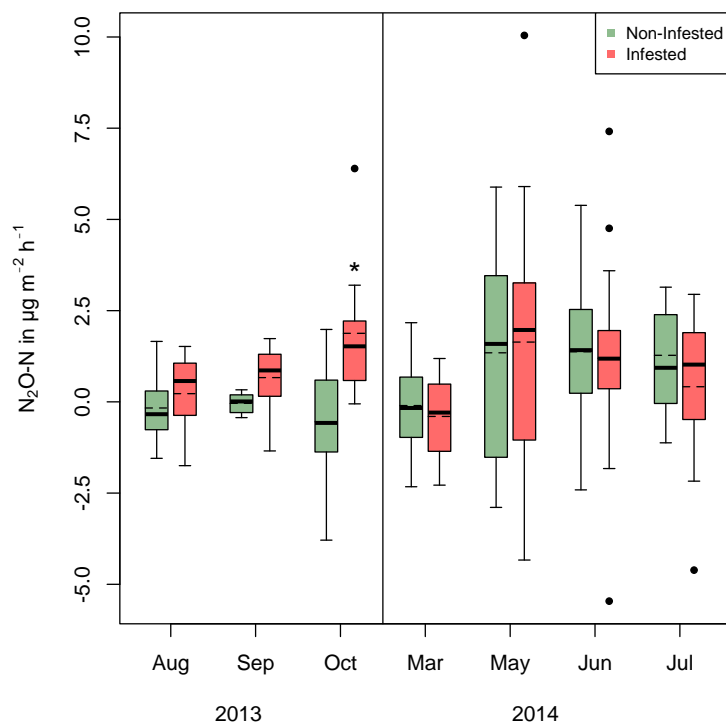


Figure 2. $\text{N}_2\text{O-N}$ emissions ($\mu\text{g m}^{-2} \text{h}^{-1}$) from the mineral soil during an outbreak of the nun moth (*Lymantria monacha* L.) in 2013 and 2014 and the adjacent noninfested control of Scots pine (*Pinus sylvestris* L.) forest sites. Infested = red, noninfested = green, Aug = August, Sep = September, Oct = October, Mar = March, Jun = June, Jul = July. Box plots show means (dotted lines) and medians (solid lines) ($n = 9$ in 2013 and 15 in 2014 for each plot). Whisker extension equals 1.5 times interquartile range distance. Asterisks indicate significant differences between infested and control plots within one sampling time (paired Wilcoxon signed-rank tests, $p \leq 0.050$).

3.2. Incubation Experiment

The accumulated carbon and nitrogen fluxes across the 31-day study period were $54.04 \pm 1.27 \text{ mg CO}_2\text{-C h}^{-1}$ in soil treatments without addition of organic matter (control), $202.11 \pm 4.39 \text{ mg CO}_2\text{-C h}^{-1}$ in soil treatments with addition of needles, and $574.83 \pm 37.85 \text{ mg CO}_2\text{-C h}^{-1}$ in soil treatments with addition of needles plus insect feces, and $731.46 \pm 7.30 \text{ mg CO}_2\text{-C h}^{-1}$ in soil treatments with addition of insect feces (Figure 3). $\text{N}_2\text{O-N}$ fluxes amounted $0.59 \pm 0.34 \mu\text{g N}_2\text{O-N h}^{-1}$ in soil treatments without addition of organic matter (control), $0.91 \pm 0.13 \mu\text{g N}_2\text{O-N h}^{-1}$ in soil treatments with addition of pine needles, $5.25 \pm 0.45 \mu\text{g N}_2\text{O-N h}^{-1}$ in soil treatments with addition of pine needles plus insect feces, and $6.14 \pm 0.27 \mu\text{g N}_2\text{O-N h}^{-1}$ emissions in soil treatments with addition of insect feces (Figure 4).

Maximum emissions of $10.98 \text{ mg CO}_2\text{-C h}^{-1}$ were reached by the insect feces treatment on the fourth day after treatment addition (with 48-fold higher emissions compared with the control). Similarly, maximum $\text{N}_2\text{O-N}$ fluxes of $0.07 \mu\text{g N}_2\text{O-N h}^{-1}$ were reached by the feces treatment at the fourth day after treatment addition (with 13-fold higher emissions compared with the control). From that day, fluxes of both gases, decreased slowly with time.

Despite similar element contents of needles and feces (see Supplementary Table S1), inputs of feces significantly accelerated soil $\text{CO}_2\text{-C}$ and $\text{N}_2\text{O-N}$ fluxes. When compared with the soil treatment without addition of organic matter (control), the experimental inputs of C and N via needles and feces accelerated $\text{CO}_2\text{-C}$ emissions 3.7-fold in treatments with addition of needles, 10.6-fold in treatments with addition of needles plus feces, 13.5-fold in treatments with addition of feces (all p -values < 0.009). $\text{N}_2\text{O-N}$ emissions were accelerated averagely 8.9-fold in treatments with addition of needles plus feces ($p < 0.010$) and 10.4-fold in treatments with addition of feces ($p < 0.010$), while it was not increased compared to the treatments with addition of needles ($p = 0.117$).

Soil C/N ratio before treatment addition 32.06 in all incubators. At the end of the experiment, C/N ratio of the control almost stayed the same with 32.08, while in the feces treatment C/N increased to 32.23. In contrast, the needle treatment and the needle plus feces treatment decreased to 31.43 and 31.46, which was significantly lower compared to the feces treatment ($p = 0.018$ and $p = 0.024$).

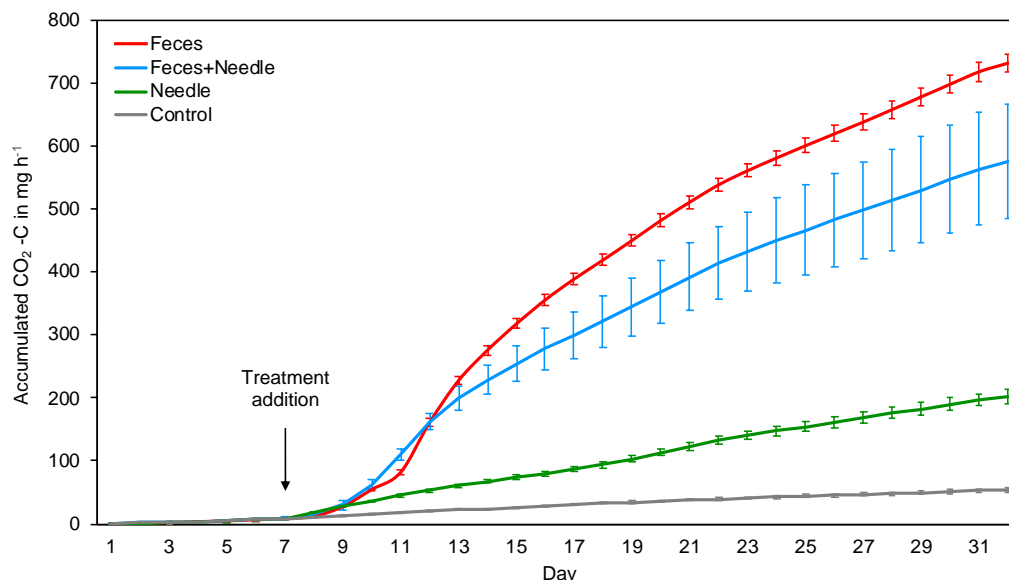


Figure 3. Accumulated $\text{CO}_2\text{-C}$ flux (mg h^{-1}) of the incubators with treatments of feces from the pine-tree lappet (*Denrolimus pini* L.), feces plus Scots pine (*Pinus Sylvestris* L.) needle litter, needle litter, and a control with soil only during the 31 days of the incubation experiment. Treatments were added on day 7 with $n = 5$. A total of 12 measurements per day were conducted.

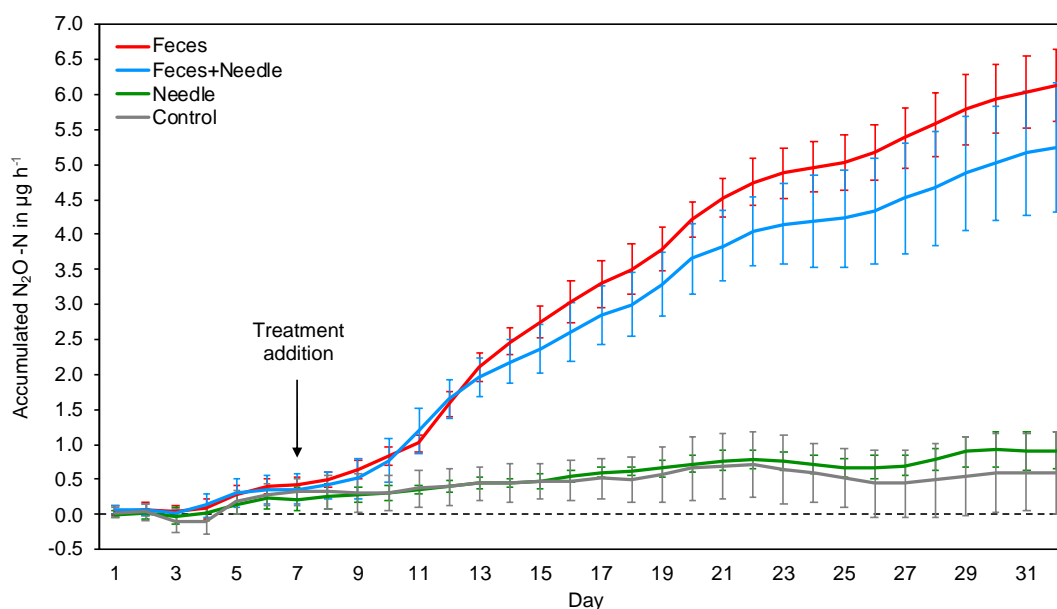


Figure 4. Accumulated $\text{N}_2\text{O-N}$ flux ($\mu\text{g h}^{-1}$) of the incubators with treatments of feces from the Pine-tree lappet (*Denrolimus pini* L.), feces plus Scots pine (*Pinus Sylvestris* L.) needle litter, needle litter, and a control with soil only during the 31 days of the incubation experiment. Treatments were added on day 7 with $n = 5$. A total of 12 measurements per day were conducted.

4. Discussion

Scots pine forests infested with the nun moth showed increased soil emissions of CO₂-C during several sampling dates and indications of increased N₂O-N emissions at one date, which both may be related to the altered quality and quantity of organic inputs during the pest outbreak. In the incubation experiment, feces input rapidly accelerated CO₂-C and N₂O-N emissions from soil with up to 14- and 25-fold, respectively, higher fluxes compared to those from needle litter. The increased deposition of organic matter during the defoliation of a pine stand provides large amounts of labile organic C and N, which in turn may positively influence microbial decomposition processes [36]. An experiment with feces from *Melanoplus borealis* F. and *Chorthippus curtipennis* F. feeding on different diets demonstrated that up to 46% of the emitted CO₂-C from soil can originate from the added feces [37]. Considering that the organic input during nun moth outbreaks can be much higher compared to those from natural (noninfested) conditions (e.g., 300% higher feces and needle litter N input on our study site in 2014 [6]), as well as the easily biodegradable structure of feces can be an explanation for the higher CO₂-C emissions from the infested forest site (even following the outbreak when the actual defoliation activity has already ceased). For example, fir forests (*Abies spec.*) defoliated by the siberian moth (*Dendrolimus superans sibiricus* Tschtrk.) showed increased rates of soil respiration even three years after the pest outbreak [38]. Therefore, increased deposition of organic matter (especially feces) during our nun moth defoliation may have contributed to the enhanced greenhouse gas emissions from forest soils during the outbreak years.

The biogeochemical pathways by which carbon is transformed and moves through forest ecosystems are strongly coupled with those of nitrogen [39]. High inputs of labile carbon enhance microbial growth and nitrogen immobilization, while low C inputs rather promote N leaching [19]. The increased soil C/N ratios of the feces treatment in our incubation experiment may therefore be an indicator of microbial immobilization, and this is supported by the relatively slow decrease of the gas emission rates following peak emissions. In contrast, C/N ratios under field conditions are often observed to decrease during insect outbreaks [20,23,24,40], even on our sampling sites [41].

The availability of organic inputs, microbial activity and emerging greenhouse gas emissions are influenced by soil aeration, fluctuation of the water table, nutrient availability, temperature, and favorable microclimatic conditions (e.g., temperature and precipitation) [42–45]. Our field study was conducted in a continental climate, with (temporally) semi-arid conditions during summer and autumn which can hamper a fast microbial decomposition [46,47]. This might have a negative impact on the microbial decomposition of organic matter during pest outbreaks, and explain the relative differences in CO₂-C and N₂O-N emissions between noninfested and infested real forests compared with those from our microcosm experiment under optimized conditions (see also [15]). Further, N₂O emissions from forest soils are spatial and temporal highly variable (“hot spots” and “hot moments” of N₂O emissions [48]), which makes measurement and comparability across sites difficult [45]. However, on our infested study site, the abundance of NO₂[−] reducers (*nirK* genes) in the soil was also found to be increased [41], indicating a genetic potential for accelerated N₂O emissions. To our knowledge, we show for the first time that both CO₂ and N₂O emissions can be triggered simultaneously by organic inputs deriving from pest insects.

Nitrification and NO₃[−] losses as well as denitrification and N₂O losses are expected to increase as the fraction of mineralized ammonium increases [49]. These processes can take place simultaneously in the same soil, e.g., in large, air-ducting pores and inside large soil aggregates, respectively [44,45,50]. Further, accelerated tree growth and increased carbon storage in biomass as well as increased autotrophic respiration from the rhizosphere and decreased heterotrophic respiration from soil microorganisms are consequences of N inputs, thereby contributing to the carbon-sink potential of a forest [39]. However, infested forest trees are often physically impaired in their N nutrition, N uptake and reduced in biomass growth rates as consequence of the defoliation [6,51,52]. Additionally, our results suggest increased microbial decomposition and CO₂ emissions during insect outbreaks. All this can have the potential to reduce the forests carbon sequestration capacity or even switch the forest to a carbon source [2–5].

5. Conclusions

Climate change appears to be a major driver of forest pest outbreaks [53–55] and most insects are expected to benefit from a high temperature to precipitation quotient [56,57]. Our results indicate that organic input during a pest outbreak in a pine forest can trigger considerable CO₂ and N₂O emissions via a transformation of tree biomass into fast decomposable organic matter, which in turn has implications for nutrient cycling and forests functioning as a carbon sink. Future studies on nitrifying and denitrifying microorganisms under variable conditions are necessary to better understand the effects of insect-derived input and the implications for soil C and N turnover processes.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/9/10/612/s1>, Figure S1: Monthly average precipitation (mm; blue bars) and mean air temperature (°C; red line) during the sampling period (August 2013–July 2014), Figure S2: Relationship between mean soil CO₂-C emissions (mg m^{−2} h^{−1}) and soil temperature (°C of the top 10 cm of soil depth), Table S1: Elemental composition of the mineral soil, Pine-tree lappet (*Dendrolimus pini* L.) feces, and Scots pine (*Pinus sylvestris* L.) needle litter used in the incubation experiment.

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Conflicts of Interest: The authors declare no conflict of interest.

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