Decreased Soil Microbial Biomass and Changed Microbial Community Composition Following a Defoliation Event by the Forest Tent Caterpillar

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Article

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Abstract: With climate change projected to increase the frequency and severity of episodic insect outbreak events, assessing potential consequences for soil microbial communities and nutrient dynamics is of importance for understanding forest resilience. The forest tent caterpillar (*Malacosoma disstria*) is an important defoliator of deciduous tree species in temperate and mixed forests of eastern North America with an invasion cycle every 10-12 years and outbreak events that can last 3-6 years. Following a defoliation episode on trembling aspen (*Populus tremuloides*) from 2015 to 2017 in Abitibi-Témiscamingue, QC, Canada, we sought to test if defoliation resulted in changes to soil bacterial and fungal communities. We hypothesized an increase in soil microbial biomass due to increased caterpillar frass inputs and potential changes in community structure following the event. Soils were sampled in July 2018, May 2019 and July 2019 from sites that had been subject to defoliation during the outbreak and from sites where no defoliation had been recorded. We assessed soil microbial biomass and fungal to total microbial activity ratio on all sampling dates, and Community Level Physiological Profiles (CLPPs) for 2018 only using a substrate-induced respiration method. Contrary to our hypothesis, we observed a significant 50% decrease in microbial biomass (μg biomass-C g^{-1} soil $hour^{-1}$) in defoliated stands suggesting tree carbon normally allocated towards root exudates was reallocated towards foliage regeneration. We noted a differentiated carbon-based substrate usage following defoliation, but no change in the fungal to total microbial activity ratio. The observed changes in the two years following the defoliation event suggest that defoliation episodes aboveground could trigger changes in soil chemistry belowground with effects on soil microbial communities that may, in turn, feedback to influence forest plant dynamics.

Keywords: forest tent caterpillar (*Malacosoma disstria*); insect outbreak; defoliation event; MicroResp; soil microbial activity

1. Introduction

Insect outbreaks are natural disturbances inherent to forest ecosystems and can alone, or coupled with droughts or fires, lead to tree mortality [1–3]. However, there is increasing concern that climate change will affect outbreak dynamics as higher global temperatures offer better conditions for the reproductive cycle of insects, causing an increase in frequency and intensity of invasive defoliator episodes [4–6]. Higher defoliating insect density may attenuate the capacity of high latitude forests to act as carbon sinks as tree growth and carbon sequestration depends in part on photosynthesis carried out by foliage [7]. Furthermore, interacting factors like drought may exacerbate tree mortality turning forests into carbon sources [8].

Consequences of altered outbreak dynamics may extend beyond host plants to soil ecosystems as vascular plants interact actively with above-ground and below-ground components of the forest ecosystem [9]. For example, insect herbivory may have both indirect and direct effects on nutrient dynamics and decomposers in soils by altering the resources flowing from above to below-ground components [9,10]. Hunter (2001) identified seven broad mechanisms by which herbivory could affect nutrient cycles. Mechanisms mediated directly by insect herbivores include increased defecation (frass) or insect cadaver inputs or changes in the nutrient content of precipitation throughfall passing through the canopy [10,11]. Indirect mechanisms mediated by host trees may include changes in leaf litter quantity and quality such as defence compounds or nutrient concentration in foliage [12], changes in the canopy structure that may modify microclimate in the understory and decreased allocation to root exudates that may alter symbiotic interactions [13]. In the medium term, changes in plant community composition may also alter nutrient cycles [10,14].

Thus, insect defoliation can reduce the amount of nutrient-rich leaves arriving to the soil while nitrogen-rich frass changes the nutritional input to the underground system, affecting carbon (C) and nitrogen (N) fluxes (eg. [15,16]). Digestion by insect herbivores reduces the C:N ratio in the frass compared to the ratio from the plant foliage [17]. This new N input is not necessarily absorbed by plant roots directly: Grüning et al. (2017) found that the new N input in the soil following a defoliation event by the nun moth (*Lymantria monacha* L.) on Scots pine (*Pinus sylvetris* L.) reduced N uptake capacity of the pine roots as the tree reallocated N from internal sources rather than increasing root N acquisition to compensate the loss of foliage. This N input could therefore become available to other organisms below-ground such as soil microbes.

Soil microbial activity, estimated typically with substrate-induced soil respiration, is an indicator of below-ground change, but studies assessing the effect of herbivorous insect outbreaks on soil microbial activity have shown mitigated results. Under artificial defoliation conditions, simulated frass addition had no effect on soil microbial activity [19]. Other laboratory studies found that litterfall and greenfall exclusion resulted in a decrease of soil microbial respiration [20], while mechanical or insect-induced defoliation increased it [15]. However, soil microbial activity following natural defoliation events generally showed a more consistent response. Multiples studies demonstrated an increase in soil microbial activity one to 16 months following an insect outbreak [11,17,21], although some found no change after 6 months [22,23] and short-term increased activity may also taper or inverse itself after three years [17].

Soil microbial community composition is increasingly studied in forest ecosystems [24], but knowledge in response to episodic insect outbreaks remains limited. Soil microbial community composition can be changed in response to recurring herbivory, as a result of changes in soil abiotic conditions such as high soil temperatures exacerbated by canopy opening during an outbreak and decreases in soil C flow from tree root exudation [25]. In response to different defoliator outbreaks, shifts of specific microbial groups have been observed. For example, Castaño et al. (2020) found a decrease in soil fungal biomass associated with a decrease in root exudates after an outbreak by the pine processionary moth. In lab cultures, Oneț et al. (2016) found an increase in fungi and a decrease in heterotrophic bacteria from soils sampled under oaks defoliated by spongy moth and attributed them to higher pH and changes in soil chemistry.

The forest tent caterpillar (*Malacosoma disstria* Hübner) is a major native defoliator of North American hardwood trees [28]. In the boreal zone, trembling aspen is its preferred host tree and the one on which the most important outbreaks have been recorded [29]. Forest tent caterpillar outbreaks occur in roughly 10-20 year cycles while many landscape variables like forest structure, climate and topography might affect the outbreak duration, lasting on average 4-6 years [30–32]. During an outbreak, the caterpillar modifies its environment by feeding on the host's foliage efficiently, typically on the deciduous canopy [33] which can negatively affect tree growth and increase mortality [34]. We sought to better understand the medium-term effects of a recent (2015-2017) outbreak of the forest tent caterpillar in eastern Canada on soil microbial communities. Sampling beneath defoliated and undefoliated aspens, we assessed soil microbial biomass and community-level physiological profiling over two successive summers following a 3-year defoliation episode (2015-2017). Given that

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soil chemistry analysis at peak defoliation in 2017 showed higher amounts of N in soil under defoliated trees from our sites attributed to frass addition (Figure S1), we hypothesized an increase in soil microbial abundance and altered community composition due to additional nutritional input from the outbreak.

2. Materials and Methods

This study was conducted in the Abitibi region of western Quebec, Canada. The study area is located within the balsam fir-white birch bioclimatic domain (Saucier et al. 2011), at and around the Lake Duparquet Research and Teaching Forest (LSRTF; 48°30′ N, 79°20′ W). Forests of the LDRTF are characterized by pure and mixed stands composed of boreal coniferous and shade-intolerant deciduous species. On mesic sites, trembling aspen (*Populus tremuloides* Michaux), white birch (*Betula papyrifera* Marshall) and jack pine (*Pinus banksiana* Lamb.) dominate early successional stands, whereas balsam fir (*Abies balsamea* L.) and eastern white cedar (*Thuja occidentalis* L.), in association with white spruce (*Picea glauca* Moench) and persistent, scattered white birch, dominate late-successional stands [35]. Glaciolacustrine clays cover 55% of the LDRTF territory [36] and are the legacy of proglacial lakes Ojibway and Barlow [37]. The climate is continental and, according to the nearest weather station (Mont-Brun), for the period 1980–2010, the growing season lasted about 150 to 160 days, whereas the mean annual temperature and mean annual precipitation was 1.0 °C and 985 mm, respectively [38].

Both the defoliated and undefoliated stands were dominated by 70–90-year-old aspens (*Populus tremuloides*), located on moderately well-drained glacial clays developed in Luvisols and separated by approximately 10 km. In 2016 and 2017, the defoliated stand was characterized by the provincial Ministry of Forests, Wildlife and Parks through aerial surveys as severe defoliation (loss of foliage all along the crown of the majority of trees) and estimated at 70 to 90% canopy loss [39,40]. Provincial surveys record no defoliation in the region in 2018 [41]. In 2017 and 2018, caterpillar colonies were counted in both stands by the research team but were only observed in the defoliated stands in 2017.

Within each stand, eight sampling sites were selected at the base of a defoliated or undefoliated *Populus tremuloides* tree. Sampled trees within the stand were separated by at least 115 m from each other. In August 2018, May 2019 and July 2019, two soil cores were extracted at 0-5 cm and 5-10 cm depth within 1 m from each selected tree and subsequently pooled. Specifically, we sampled 745 ml of fresh soil that was sieved on a 2 mm test sieve (Retsch), put in sterile tubes, transported in a cooler with multiple ice packs and then frozen at -20 °C until analysis.

Using the substrate-induced soil respiration method of MicroResp™ [42] and FungiResp [43], both total microbial biomass (μg CO_2-C g^{-1} $hour^{-1}$) and the fungal ratio were tested as indicators of soil microbial activity. Soils were defrosted for twenty-four hours before being adjusted with distilled water to a water-holding capacity of 35% for all samples. After a week of incubation, bronopol then glucose was added to the samples and after six hours, the colorimetric microplate was read using a microplate spectrophotometer (Multiskan Go, Thermoscientific). A factor of 40 of glucose-induced respiration (GIR) was used as a proxy of microbial biomass [43,44]. We estimated the fungal ratio of our soils by adding a solution of bronopol at 78 μg g^{-1} of soil as a bacterial inhibitor (Sassi et al.,2012). A ratio of the microbial activity (μg CO_2-C g^{-1} $hour^{-1}$) induced with the inhibitor divided by the microbial activity induced with glucose allowed us to estimate the proportion of fungi compared to bacteria in our samples. Calibration curves associating percentages of CO_2 to different absorbances of the detection plate were determined with the two different indicator solutions using a portable CO_2 analyzer (EGM-5, PP Systems). This allowed us to convert the normalized 6-hour data to %CO_2. While 8 soil samples from the 0-5 cm depth were taken from each stand type at each of the three sampling dates, 9 of the 48 samples were compromised in the lab due to the over-adjustment of water saturation capacity in very wet samples. Ultimately, this resulted in variable replication (n = 5 to 8 samples) depending on treatment and sampling date (see caption in Figure 1 for explicit details). The 5-10 cm depth was only analyzed for soils collected in August 2018.

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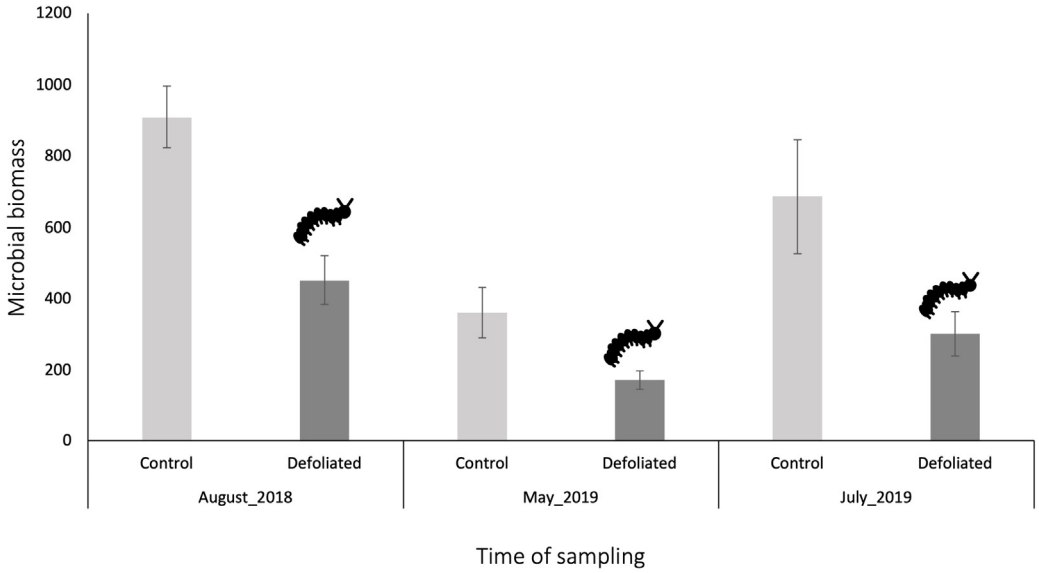

Figure 1. Mean microbial biomass (µg biomass-C g^{-1} soil $hour^{-1}$) ± standard error from glucose-induced respiration of the top 5 cm of soil sampled under undefoliated control (N = 8, 6, 6) and defoliated (N = 7, 5, 7) *Populus tremuloides* trees. Soils were sampled in August 2018, May 2019 and July 2019 in the Lake Duparquet Research and Teaching Forest (Abitibi, QC).

The technique of assessing community-level physiological profiles (CLPPs) measures the ability of the soil microbiome to metabolize different carbon substrates. To determine the functional composition of the microbial communities by CLPPs, we created a catabolic fingerprint by testing four dissolved carbon sources ecologically relevant to induce differentiated mineralization between soil microbial communities [45]. Two carbohydrates (fructose and glucose), one amino acid (glycine) and one carboxylic acid (malic acid) providing different chemical types of acids and sugars were selected following Campbell et al. (2003) and Sassi et al. (2012). In the same manner as the MicroResp ™ protocol, soils were defrosted for twenty-four hours before being adjusted with distilled water to a water-holding capacity of 35% for all samples. After a week of incubation, we added the four different substrate solutions (30 mg g^{-1}). Aliquots of 25 µL per solution were inserted in each corresponding well of the MicroResp™ 96-deep-well plate according to Sassi et al. (2012). After six hours, the colorimetric microplate was read using a microplate spectrophotometer (Multiskan Go, Thermoscientific). Only 2018 samples were analyzed. To compare substrate breakdown from each treatment, relative utilization was assessed by the proportion of the total respiration induced by each substrate. To calculate the relative utilization by the microbial community for each defoliation treatment, the mean respiration per substrate per treatment was divided by the mean of total respiration across substrates per treatment.

Soil microbial biomass and the relative utilization of the four carbon-based substrates were analyzed with a mixed model analysis where defoliation and time of sampling were fixed effects and the individual tree beneath which the soil was sampled (site) was a random effect. The mixed model analysis was done with lme4 [46] and lmerTest [47] libraries. Analyses were done in the Rstudio (v.1.2.1335) (Rstudio Inc., Boston, USA) environment with R software (v.3.6.0) (R Development Core Team, Vienna, Austria).

3. Results

3.1. Soil microbial biomass

Soil microbial biomass ranged from 173 to 911 µg biomass-C g^{-1} soil $hour^{-1}$ (Figure 1, $P < 0.001$), with lower biomass in the spring and higher biomass in the late summer, but no significant interaction between defoliation and sampling date was observed (Figure 1, $P = 0.36$). When assessing

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glucose-induced activity, microbial biomass in the top 5 cm of soil from previously defoliated stands was consistently about half that in control stands, across all sampling dates (Figure 1, $P < 0.001$). This microbial biomass decrease was also observed for all other substrates (glycin, fructose and malic acid) included to assess the catabolic fingerprint (August 2018 only) with each substrate-induced activity being two to three times the magnitude in control compared to defoliated stands (Figure 2, $P < 0.001$). Furthermore, there was no significant interaction between defoliation and the type of substrate used (Figure 2, $P = 0.50$).

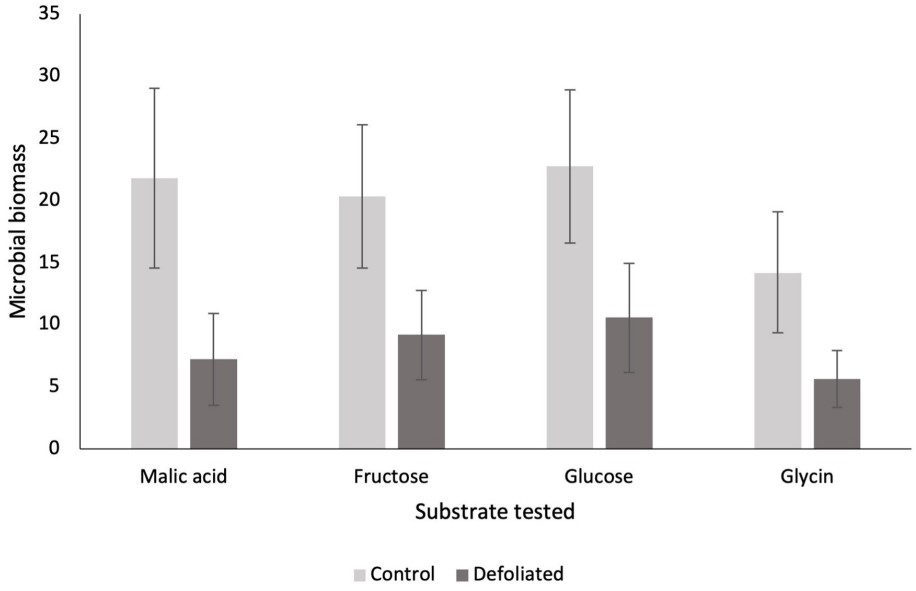

Figure 2. Mean microbial respiration (μg biomass-C g^{-1} soil $hour^{-1}$) ± standard error from carbon-based substrate-induced respiration of the top 5 cm of soil sampled under undefoliated control (N = 8) and defoliated (N = 7) *Populus tremuloides* trees. Soils were sampled in August 2018 in the Lake Duparquet Research and Teaching Forest (Abitibi, QC).

In contrast, average soil microbial biomass in deeper soils (5-10 cm depth, analyzed for 2018 only) showed no effect of defoliation ($P = 0.31$). However, average soil microbial biomass in deeper soils was ca. 50% (65% for non-defoliated and 37% for defoliated sites) lower than that measured in the 0-5 cm layer (data not shown).

3.2. Soil microbial community

Community-level physiological profiles (CLPP) offer an indicator of microbial community functional composition. Figure 3 reshapes the data shown in Figure 2, offering a view of the proportion of respiration from each substrate relative to the total respiration induced across all substrates. Relative respiration of our microbial communities showed a differentiated usage of four carbon-based substrates between the control and the defoliated stands from soil sampled in 2018 (Figure 3). The defoliated stand microbial community used significantly less malic acid than that from soil under control trees but used more carbohydrates-based substrates (Figure 3). Relative respiration of carbohydrate substrates (glucose and fructose) was on average 12 $\pm$ 9% higher for the defoliated treatments compared to control treatments ($P < 0.05$). Inversely, respiration from the carboxylic acid substrate (malic acid) was 20% lower for the defoliated treatment than the control ($P < 0.05$).

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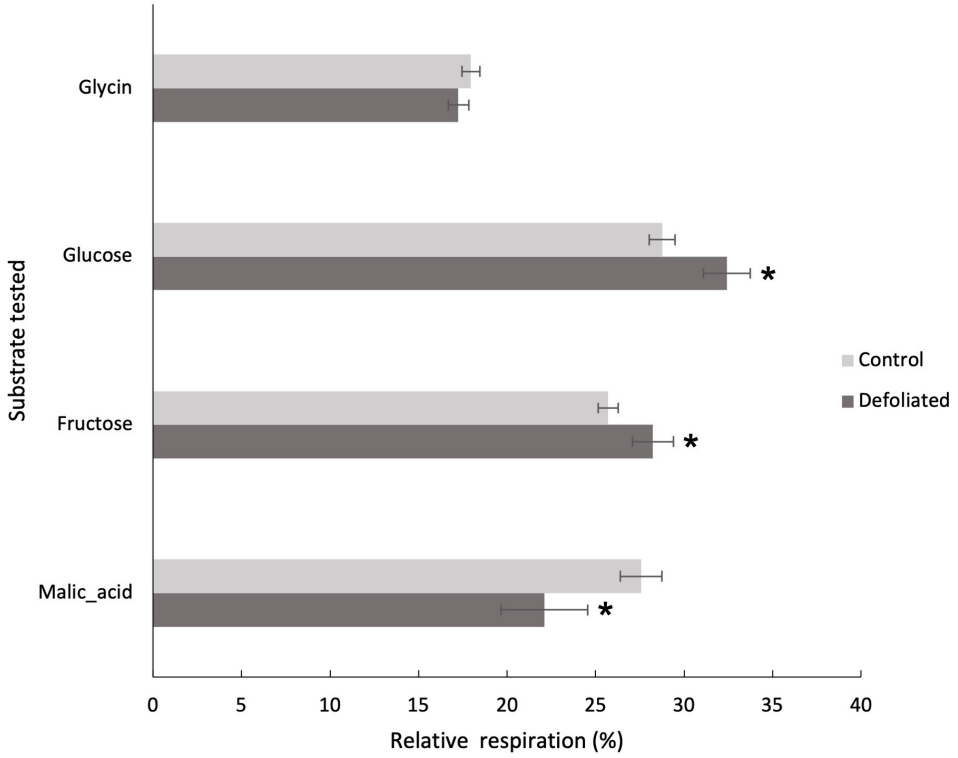

Figure 3. Relative respiration with ± standard error from four different carbon substrate-induced respiration of the top 5 cm of soil under undefoliated control (N = 8 white) and defoliated (N = 6 black) *Populus tremuloides* trees. Soils were sampled in August 2018 in the Lac Duparquet Research and Training Forest (Abitibi, QC).

3.3. Fungal ratio

The average fungal to total microbial activity ratio across defoliated and control plots was 0.86 ± 0.17, revealing a soil dominated by fungi throughout the samples. There were no significant differences between defoliated and control plots (P = 0.37).

4. Discussion

Our two-year study following a severe defoliation outbreak of the forest tent caterpillar provided us with a medium-term understanding of microbial dynamics in the trembling aspen-dominated stands of the mixed wood boreal forest. Most studies to date have been limited to short-term responses [15,19,20,22,23,33,48] or drawn general conclusions using artificial defoliation events [15,19,20,49]. While these latter artificial defoliation experiments have helped understand valuable mechanisms, such study systems are limited in their ability to reproduce the complex interactions of natural outbreaks. Our results showed that, contrary to our hypothesis that N input by frass would stimulate microbial activity [15,17,18,21], microbial biomass decreased by half in defoliated sites in the top 5 cm of soil and the response was consistent over all sampling dates (Figure 1) and tested substrates (Figure 2). As hypothesized, we observed a shift in microbial functional composition in defoliated sites based on assessments with selected substrates (Figure 3), but no change in the overall fungal activity was observed. Potential interpretations for our results include changes in the quality of aspen foliage [50], a decrease in C allocation to roots [13] and a change in vegetation regeneration benefiting balsam fir over trembling aspen [34].

Our observed decreased microbial response may be explained by changes in tree foliage quality following the major defoliation event, resulting in afterlife effects of tree litter [5,6]. A major forest tent caterpillar outbreak can lead to altered foliage quality in the second flush of leaves of host tree species with lower nitrogen and increased defence compounds. Trembling aspens produce foliar phenolic glycosides as a chemical defence in response to herbivory which is an effective deterrent to

defoliators [12]. This effect can persist in time with foliage grown two years after a defoliation event having on average six times more phenolic glycosides than before defoliation (Donaldson and Lindroth 2008). Condensed tannins can also increase in remnant defoliated leaves (Donaldson and Lindroth 2008). When leaf litter falls, condensed tannins derived from plant material can form complexes with proteins [51] binding N [52,53] and chitin from fungi [54,55]. Such complexes, particularly with high molecular mass tannins, can inhibit microbial activity in soil by binding extracellular substrates [54,56,57]. Several short-term (<1 year) studies on microbial activity found an increase [11,15,17,21] or no change following insect herbivory [23,48]. However, two years after a natural defoliation event which is more similar to conditions in this study, Streminska et al. (2006) found a decrease in microbial biomass explained by lower amounts of litterfall. In her meta-analysis on N addition and microbial biomass in soil across multiple ecosystem types and biomes, [58] also found an overall decrease in microbial biomass with increasing amounts of N, particularly in studies of longer duration, indicating soil microbes are not necessarily N limited.

An alternate or complementary interpretation of our reduced microbial biomass following the outbreak event is that insect herbivory can induce an indirect response from the host tree by altering C allocation to tree roots and thus affecting microbial activity in soils. Repeated herbivory, by reducing autotrophic C supply, can lead to reduced root activity [59] and fine root biomass [60] implying that trees allocate less C below-ground than before the defoliation event. In their meta-analysis on below-ground responses to insect herbivory in ecosystems with woody plants, [13] showed that defoliators decrease carbon allocation from the host plant to its roots, provoking a decrease in root biomass and exudation. Following a disturbance, trembling aspen commonly regenerates via root suckering, but can be limited by competition from other trees [61]. We would therefore expect aspens not only to allocate C to their foliage regeneration instead of their roots but also to reduce their investment to suckering because of the competition from balsam fir that can benefit from the outbreak [34].

The forest tent caterpillar outbreak, by favouring regeneration of certain tree species such as balsam fir, could help explain the differences in CLPPs between our defoliated and undefoliated sites with soil microbial communities composition altered following a change in the availability of their preferred carbon source and soil chemistry. The caterpillar outbreak can benefit competing tree species in the forest that may ultimately alter vegetation dynamics favouring coniferous over deciduous trees as the dominant litter type changes on the forest floor [34]. The CLPPs profiles differed from soil located beneath defoliated and undefoliated control trees, indicating that a change in litter identity from aspens to firs could stimulate different microbial communities able to degrade coniferous or deciduous litter types. Light availability allowed by canopy openings following a defoliation episode can contribute to changes in the plant community composition, facilitating the growth of shade-intolerant species [10]. At the same mixed wood site in Abitibi-Témiscamingue QC, Moulinier et al. (2013) showed previous outbreaks benefited the regeneration of a conifer *Abies balsamea* (Balsam fir) to the detriment of the deciduous trembling aspen.

Furthermore, the CLPPs of soils located beneath defoliated trees indicated that those microbial communities used less carboxylic acid (malic acid) and more monosaccharides (fructose and glucose) than under undefoliated trees (Figure 3). Studies comparing soils from deciduous and coniferous forests also found microbes from the latter were less efficient at using carboxylic acids [63,64]. However, Chodak et al. (2016), found less efficient use of monosaccharide substrates by microbial communities from the coniferous forest. Along with a change in vegetation regeneration in the defoliated forest, a decrease in root biomass and root exudates of labile C in the topsoil can also shift the microbial communities associated with roots, as illustrated by a negative correlation between herbivory and ectomycchorizal fungi abundance [13,60]. Other studies have suggested higher soil acidity [27] or lower C: N ratio in the litterfall [17] could increase fungal biomass in topsoil under defoliated trees compared to undefoliated trees, but our fungal ratio indicator remained constant between undefoliated and defoliated sites. This possible change in forest regeneration at our site favouring balsam fir over trembling aspen may also have consequences for soil micronutrients. In 2017 at the peak of the outbreak, higher N, P, K, Ca and Mg concentrations were found in soils under

defoliated compared to non-defoliated trees (Figure S1, $p<0.005$). For example, Ca concentrations were double at defoliated sites than at undefoliated sites which could reflect a change in nutrient absorption by dominant trees. Trembling aspen have very high Ca requirements [65] and thus gradual replacement by other species would explain the high concentrations of Ca on soil under defoliated trees.

5. Conclusions

Although the recent meta-analysis by Kristensen et al. (2020) did not report significant effects of outbreak herbivory on forest microbes, their analysis mixes outbreaks on both coniferous and deciduous tree species with deciduous stands being largely underrepresented in the 60 articles analyzed. Our study therefore suggests that microbes living in deciduous stands of mixedwood boreal forests may respond differently from those in coniferous forests, which could eventually feedback on vegetation dynamics in a warming climate where outbreaks are projected to become increasingly common. This study underlines the importance of considering soil microbes as part of a forest ecosystem perturbation's impacts such as an insect defoliation event.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Soil chemistry analysis;

Author Contributions: Conceptualization, EDM. and TH; methodology, EDM and TH; resources, TH and ED; data curation, EDM; writing—original draft preparation, EDM and TH; writing—review and editing, ED; supervision, TH; project administration, TH; funding acquisition, TH and ED.

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Conflicts of Interest: The authors declare no conflict of interest

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