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Carbon and Nitrogen Responses in Litterfall and Litter Decomposition in Red Pine (*Pinus densiflora* S. et Z.) Stands Disturbed by Pine Wilt Disease

Choonsig Kim^{1,*}, Seongjun Kim², Gyeongwon Baek¹ and A-Ram Yang³

- ¹ Department of Forest Resources, Gyeongnam National University of Science and Technology, Jinju 52725, Korea; kwb1926@naver.com
- ² Institute of Life Science and Natural Resources, Korea University, Seoul 02841, Korea; dao1129@hanmail.net
- ³ Forest Technology and Management Research Center, National Institute of Forest Science, Pocheon 11186, Korea; aryang@korea.kr
- * Correspondence: ckim@gntech.ac.kr; Tel.: +82-55-751-3247

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Abstract: *Research Highlight:* Forest disturbance by insects or disease can have a significant influence on nutrient return by litterfall and decomposition, but information regarding disturbance gradients is scarce. This study demonstrated that the disturbance intensity caused by pine wilt disease greatly altered the quality and quantity of carbon (C) and nitrogen (N) in litterfall components and decomposition processes. Background and Objectives: This study was conducted to evaluate the C and N status of litterfall and litter decomposition processes in a natural red pine (Pinus densiflora S. et Z.) stand disturbed by pine wilt disease in southern Korea. Nine red pine plots with varying degrees of disturbance caused by pine wilt disease were established based on differences in the stand basal area. Litterfall and the decomposition of needle litter and branches under different degrees of disturbance were measured for three years. *Results*: There was a significant correlation (p < 0.05) between disturbance intensity and the C and N concentration of litterfall components depending on the time of sampling. The annual C and N inputs through litterfall components decreased linearly with decreasing disturbance intensities. The decomposition rates of branches were higher in slightly disturbed plots compared with severely disturbed plots for the late stage of branch decomposition, whereas the decomposition rates of needle litter were not affected by the disturbance intensity of pine wilt disease. Carbon and N concentrations from needle litter and branches were not linearly related to the intensities of disturbance, except for the initial stage (one year) of needle litter decomposition. *Conclusions*: The results indicated that the incidence of pine wilt disease was a major cause of C and N loss through litterfall and decomposition processes in pine wilt disease disturbed stands, but the magnitude of loss depended on the severity of the disease disturbance.

Keywords: canopy removal; nutrient dynamics; sanitation thinning; carbon and nutrient cycle; pine wilt disease

1. Introduction

Litterfall inputs and decomposition are an important pathway for nutrient returns in forest ecosystems because significant amounts of organic matter and nutrients in the soil can be transferred by litterfall and litter decomposition processes [1]. However, forest disturbance by epidemic outbreaks of insects or disease can have a significant influence on nutrient returns as the death or removal of damaged trees causes changes in biotic and abiotic factors [2–4]. For example, nutrient inputs caused by litterfall are linearly decreased with increased intensities of forest disease [5], whereas the decomposition rates of needle litter are increased [6], unchanged [7], or decreased [8,9] with the degree of tree removal.



The incidence of pine wilt disease, a highly destructive forest disease in the world [10,11], can have considerable effects on nutrient cycling by litterfall and decomposition processes due to the death or damage of trees. For example, pine wilt disease and the associated management practices generate canopy caps and elevate light availability and temperature [12], which possibly stimulates the decomposition process on the forest floor [4,13]. They also affect soil properties, such as water-holding capacity, and other influential factors affecting litter decomposition. The mortality of the infected trees leads to a decrease in litterfall as a result of the reduced tree density [5]. In addition, wood debris, such as dead logs, branches, and stumps, collects on the forest floor during the removal of infected trees; the removal of infected trees is one of the most important control strategies for pine wilt disease disturbed forests in Korea [5,14]. However, since information along the gradient of infection is scarce, it is difficult to evaluate the quantitative changes in nutrient inputs through litterfall and decomposition processes because of the spatial variation of infected pines in small-scale stands [12,15].

The incidence of pine wilt disease is likely to modify carbon (C) and nitrogen (N) inputs through litterfall and litter decomposition because the clearcutting or selective cutting of infected pine trees is the only option currently available for controlling the spread of pine wilt disease in Korea [14,16]. Thus, litterfall and litter decomposition processes in pine wilt disease stands needs to be examined across different disease disturbance intensities. The objectives of this study were to: (1) quantify the C and N inputs through litterfall and (2) determine the patterns of C and N release from decomposing needles and wood litter at varying disturbance intensities due to pine wilt disease. Given the reduction in tree density and changes in microclimate in infected and managed forests, our study hypothesized that an intensive removal of infected trees would decrease C and N inputs through litterfall and promote C and N release through litter decomposition compared with a partial removal.

2. Materials and Methods

2.1. Study Site and Experimental Design

This study was conducted in approximately 40-year-old natural red pine stands ($35^{\circ}12'21''$ N, $128^{\circ}10'24''$ E, 150 m) in the Wola National Experimental Forest, which is one of the most severe pine wilt disease-disturbed forests in Korea [5,12]. The average annual precipitation and temperature in this area are 1490 mm yr⁻¹ and 13.1 °C, respectively. The soil is a slightly dry dark-brown forest soil (Inceptisol, USDA Soil Taxonomy) originating from sandstone or shale, with a silt loam texture. The site index in dominant pine trees indicated a low forest productivity (site index, 8 at 20-year-old base age). The understory tree species include *Juniperus rigida* S. et Z., *Lespedeza* spp., *Lindera glauca* Blume, *Quercus variabilis* Blume, *Q. serrata* Thunb., *Robinia pseudoacacia* L., and *Smilax china* L. More information on the study sites is available in References [5,12].

The experimental design consisted of 10 m \times 10 m plots that were established in stands with different degrees of disturbance induced by selective cutting of the infected and dead pine trees on a small scale (Figure 1). The plots were established on similar-facing slopes and aspects to minimize spatial variations and to reduce sampling bias of site components. In addition, sampling schemes were repeated yearly to interpret the consistency of treatment effects because different degrees of disturbance due to pine wilt disease were not true replicates [17]. Thus, the results from this study need to be interpreted cautiously, although study sites were representative of pine wilt disease forests in southern Korea. The stand basal area was highest (35.9 m² ha⁻¹) at 2100 tree ha⁻¹ and lowest (4.2 m² ha⁻¹) at 300 tree ha⁻¹ (Table 1).

2.2. Litterfall

Three circular litter traps (a surface area of 0.25 m²) within each plot were installed 60 cm above the forest floor in each plot (total, 27 litter traps) to quantify litterfall in stands with different degrees of disturbance due to pine wilt disease (Figure 1). Litter was collected thirteen times (27 July, 18 September, 22 October, 18 November, 23 December 2009; 28 March, 20 May, 20 July, 17 September, 26 October,

11 November, 10 December 2010; and 13 April 2011) between 29 May 2009 and 13 April 2011. Litter from each trap was transported to the laboratory and oven dried at 65 °C for 48 h. All dried samples were separated into needles, bark, cones and flowers, branches, and miscellaneous components, and each portion was weighed. The litter samples were combined based on their collection time (July–November, December–June) each year because some components of litter samples in severely disturbed plots were too limited for chemical analysis. The composite litter samples were ground through a 40-mesh (0.425 mm) stainless steel sieve, and C and N concentrations were determined using an elemental analyzer (Thermo Scientific, Flash 2000, Milan, Italy). Total C and N inputs, which are defined as the absolute amount of C and N return through litterfall components, were calculated by multiplying the weight of each litterfall component by the C and N concentrations.

Disturbance Intensity	Basal Area (m ² ha ⁻¹)	Tree Density (tree ha ⁻¹)	Mean DBH (cm)	Mean Height (m)
Slight	35.9	2100	14.08 (6.0-21.5) *	9.57 (7.40–10.66)
Slight	35.5	1800	15.40 (9.5-21.0)	9.66 (8.30-10.35)
	33.1	2500	12.60 (8.0-18.9)	10.74 (9.21-11.87)
	28.6	1300	16.36 (11.0-23.0)	10.30 (9.06-11.36)
	25.3	1500	14.22 (8.1–19.3)	10.82 (10.36-11.32)
	14.3	1100	12.56 (9.7-17.0)	8.42 (7.36-9.52)
	13.9	800	14.54 (11.2–21.6)	8.14 (7.28-9.19)
•	9.7	500	15.36 (10.8–19.9)	8.25 (6.84–9.88)
Severe	4.2	300	13.23 (12.0–14.4)	7.36 (5.93–8.66)

 Table 1. Stand characteristics in pine wilt disease disturbed stands.

DBH, diameter at breast height (1.2 m). * Mean (minimum-maximum).



Figure 1. Location of the study site in pine wilt disease disturbed stands. (a) Slightly disturbed stand; (b) moderately disturbed stands; (c) severely disturbed stands; (d) litter trap; (e) needle litterbag; and (f) branch bag.

2.3. Decomposition of Needles and Branches

The decomposition rates of needle litter were measured using the litterbag technique (Figure 1). Fresh needle litter from each treatment was collected from the forest floor on 10 December 2009, which followed a concentrated shedding event during autumn. After collection, the litter was air-dried at room temperature for 10 days. Then, a sample of 10 g was weighed and placed in 30 cm \times 30 cm nylon net bag with a mesh size of 0.1 mm. Subsamples from the litter were also taken to determine the dry weight after drying in an oven at 65 °C for 48 h. Six litterbags (total 54 bags) for each plot were randomly placed on the forest floor on 23 December 2009. The litterbags were collected after

352 days (10 December 2010) and 1103 days (15 March 2013) from each plot during the study period. Each litterbag sample was then oven dried at 65 °C for 48 h, and the weight loss rates were determined. All samples in the litterbag were ground in a Willy mill to pass through a 0.04 mm mesh. An elemental analyzer (Thermo Scientific, Flash 2000, Milan, Italy) was used to quantify the C and N concentrations in the ground materials. The remaining C and N in the bags was estimated by multiplying the weight of undecomposed litter and the C or N concentration.

The decomposition rates of wood litter were measured from fresh pine branches collected from the study sites in June 2009. The pine trees were felled from the study site with hand saws. Fifty-four branch log sections (50–60 mm diameter and 150–160 mm long) were prepared and oven dried at 65 °C for 7 days. Then, a branch sample was weighed and placed in a 30 cm \times 30 cm nylon net bag with a mesh size of 0.1 mm. Six branch decomposition bags were randomly installed on the forest floor in each treatment plot on 28 June 2009 (Figure 1). Branch decomposition bags (total 54 bags) were collected after 358 days (21 June 2010) and 1353 days (15 March 2013). The collected bags were oven dried at 65 °C for 7 days, cleaned by gentle brushing with a soft paintbrush to remove the mineral soil, and weighed to determine branch decomposition rates. All branch samples were ground in a Willy mill to pass a 0.04 mm mesh sieve, and the C and N concentrations in the ground materials were determined by an elemental analyzer (Thermo Scientific Flash 2000, Milan, Italy). The remaining C and N in the bags was estimated by multiplying the weight of undecomposed branch samples and the C or N concentration.

2.4. Soil Property

Soil bulk density at the surface depth (5–10 cm) was determined after drying at 105 °C from soil samples collected in 100 cm³ stainless steel cans. Soil samples were collected four times at a depth of 20 cm using a 2 cm diameter stainless steel sampling probe (Oakfield soil sampler) between June and September 2009 to measure the soil nutrient concentration. Before chemical analyses, the soil samples were sieved through a 2 mm mesh to separate coarse rock from the total soil. Soil C and N concentrations were determined using an elemental analyzer (Thermo Fisher Scientific Flash 2000, Milan, Italy). Soil P concentration extracted by NH₄F and HCl solutions [18] was determined using a UV spectrophotometer (Jenway 6505, Staffordshire, UK). Soil exchangeable cations (K⁺, Ca²⁺, and Mg²⁺) extracted by NH₄Cl solution [18] with a mechanical vacuum extractor (Model 24VE, SampleTek, Science Hill, KY, USA) were determined through ICP-OES (Perkin Elmer Optima 5300DV, Shelton, CT, USA).

To evaluate the relationship between needle or branch decomposition and soil environmental characteristics, three soil samples from each plot were collected monthly during the study period at a depth of 20 cm using an Oakfield soil core sampler. The soil samples were transported to the laboratory and oven dried at 105 °C for 48 h to measure the soil gravimetric water content. The soil pH (1:5 soil:water) was measured using a glass electrode (ISTEC Model pH-220 L, Seoul, Korea). Soil temperature was measured at a depth of 8 cm adjacent to the soil collection sites using a soil temperature probe (K-type, Summit SDT 200, Seoul, Korea).

2.5. Data Analysis

Pearson's correlation analyses were performed to evaluate the relationship between the C and N concentrations of litterfall components (i.e., needles, branches, bark, cones and flowers, miscellaneous, and total litterfall) and the various levels of basal area [19]. Regression analyses were used to determine the relationships between the different disturbance intensities (independent variables) and the C and N inputs through the litterfall components, decomposition of needle litter, and decomposition of branches (dependent variables). This study utilized basal area as an indicator of the disturbance intensity, given that the basal area of each plot could reflect the number of undamaged trees by pine wilt disease and the following selective cutting. In addition, the responses of litterfall and litter decomposition were linearly linked to various levels of basal area following tree removal [20,21]. A *p* value < 0.05 was considered statistically significant.

3. Results

3.1. Soil Property

There was considerable variability in soil bulk density and exchangeable cation (Ca^{2+} , Mg^{2+}) concentrations in the different disturbance intensities, whereas soil chemical properties, such as organic C and total N, were not significantly different among different disturbance intensities of pine wilt disease (Table 2). The soil fertility levels such as available P and exchangeable Ca^{2+} and Mg^{2+} concentrations showed declining patterns with an increased disease disturbance intensity.

3.2. Carbon and Nitrogen Responses of Litterfall

The C concentration of litterfall components was significantly correlated with the disturbance intensity of pine wilt disease (e.g., various levels of the basal area) depending on the time of sampling, except for miscellaneous litter (Figure 2). In contrast to the C concentration of litterfall components, the N concentration of needle litter was generally higher in the slightly disturbed plot in the high basal area than in the severely disturbed plots in the low basal area (Figure 2). However, the N concentration of other litterfall components, such as branches, bark, cone and flower, and miscellaneous litter, was not affected by disturbance intensity, except for one sample of miscellaneous litter (Figure 2).



Figure 2. Correlation between carbon and nitrogen concentration (n = 3) in litterfall components [(**a**,**b**) needle, (**c**,**d**) branches, (**e**,**f**) bark, (**g**,**h**) cones and flowers, (**i**,**j**) miscellaneous] and basal area in pine wilt disease disturbed stands. Vertical bars represent standard error. ns: non-significance.

Basal Area	Basal AreaBulk DensityCompared to $(m^2 ha^{-1})$ Compared to $(g cm^{-3})$	Coarse Fragment	Organic C (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N Ratio	Available P (mg kg ⁻¹)	Exchangeable (cmolc kg ⁻¹)		
$(m^2 ha^{-1})$		$(g g^{-1})$					K+	Ca ²⁺	Mg ²⁺
35.9	1.15 (0.03)abc	0.58 (0.02)a	31.6 (7.5)a	1.7 (0.4)a	19.4 (1.0)b	2.4 (0.2)abc	0.12 (0.01)a	3.32 (0.25)a	1.93 (0.15)a
35.5	1.12 (0.04)abc	0.42 (0.01)a	26.9 (3.6)a	1.4 (0.2)a	19.9 (1.4)b	2.8 (0.8)ab	0.16 (0.01)a	3.83 (0.20)a	1.60 (0.24)ab
33.1	1.29 (0.05)ab	0.42 (0.01)a	24.5 (5.3)a	1.3 (0.3)a	19.1 (1.2)b	1.8 (0.3)bcd	0.14 (0.02)a	3.03 (0.17)ab	1.21 (0.10)b
28.6	1.11 (0.03)abc	0.45 (0.03)a	32.7 (9.2)a	1.7 (0.4)a	18.7 (0.6)b	3.9 (0.8)a	0.16 (0.01)a	4.02 (0.20)a	1.62 (0.13)ab
25.3	1.32 (0.05)a	0.46 (0.05)a	14.4 (3.6)a	0.7 (0.2)a	20.6 (1.3)b	1.6 (0.2)bcd	0.13 (0.02)a	2.95 (0.23)ab	1.48 (0.05)ab
14.3	1.03 (0.05)bc	0.48 (0.04)a	21.8 (5.1)a	0.8 (0.2)a	34.3 (6.7)a	0.7 (0.2)cd	0.10 (0.02)a	1.10 (0.16)c	0.58 (0.02)c
13.9	1.03 (0.04)bc	0.40 (0.09)a	33.7 (7.6)a	1.3 (0.3)a	25.8 (1.1)ab	0.9 (0.1)cd	0.09 (0.02)a	1.17 (0.19)c	0.52 (0.09)c
9.7	1.08 (0.03)abc	0.55 (0.04)a	32.2 (7.4)a	1.3 (0.3)a	24.5 (2.1)ab	0.8 (0.1)cd	0.29 (0.12)a	1.63 (0.18)bc	0.63 (0.03)c
4.2	1.00 (0.06)c	0.47 (0.06)a	27.0 (6.4)a	1.3 (0.3)a	22.0 (0.7)b	1.0 (0.1)bcd	0.17 (0.02)a	2.60 (0.83)abc	0.63 (0.05)c

Table 2. General soil properties in pine wilt disease disturbed stands (n = 3).

Values (n = 3) in parentheses are standard error. The different letter among each basal area treatment represent a significant difference at p < 0.05.

The linear regression analyses developed for the C and N inputs through litterfall components were significant ($r^2 = 0.42-0.91$; p < 0.05), except for the C input in branch litter sampled in 2010–2011 (Figure 3). The C and N inputs through litterfall components decreased with decreasing basal area due to considerable differences in the basal areas. The best fit with the highest coefficient of determination (r^2) was generally obtained for total litterfall, followed by needles, branches, miscellaneous, bark, and reproduction litter.



Figure 3. Relationships between basal area and carbon and nitrogen inputs through litterfall components [(a,b) needle, (c,d) branches, (e,f) bark, (g,h) reproduction, (i,j) miscellaneous, (k,l) total] in pine wilt disease disturbed stands (*n*= 3). Vertical bars represent standard error.

3.3. Weight Loss and Carbon and Nitrogen Status during Decomposition Processes

The weight loss rates of needle litter were not linearly related to the basal area for two sampling times (Figure 4), but the weight loss rates of branches sampled at 1353 days (Figure 5) linearly increased with increasing basal area ($r^2 = 0.90$, p < 0.01). The C concentration of decomposing needle litter and branches was not affected by the basal area, but the N concentration, C/N ratio, and remaining C and N of needle litter were linearly related to the basal area during the initial incubation year (358 days).

In contrast to needle litter, the remaining C and C/N ratios of branches were linearly related to the basal area during the late incubation (1353 days).



Figure 4. Relationships between basal area and decomposition of needle litter [(**a**) remaining mass, (**b**) carbon and (**c**) nitrogen concentration, (**d**) C/N ratio, (**e**) remaining C and (**f**) N] in pine wilt disease disturbed stands (n = 3). Vertical bars represent standard error.



Figure 5. Relationships between basal area and decomposition of branches [(**a**) remaining mass, (**b**) carbon and (**c**) nitrogen concentration, (**d**) C/N ratio, (**e**) remaining C and (**f**) N] in pine wilt disease disturbed stands (n = 3). Vertical bars represent standard error.

4. Discussion

4.1. Carbon and Nitrogen Inputs through Litterfall

The C concentration of each litterfall component was generally unaffected by the disturbance intensity (represented by basal area). However, the N concentration of needle litterfall decreased with decreased basal area (r = 0.74-0.88, p < 0.05), indicating low soil N availability in severely disturbed plots since the N concentration of needle litter was associated with the soil N status at the sites [22]. For instance, the C/N ratio, which is one of the indicators of soil quality [23], was higher in severely

disturbed plots (22.0–25.8) than in slightly disturbed plots (19.1–19.9). This result supported that the C concentration of needle litterfall is generally greater in poor-quality sites due to the low nutrient concentration of needle litter compared with trees grown in better environmental conditions [24]. However, the N concentration of branch or bark litter was not significantly correlated (p > 0.05) by the disturbance intensity caused by pine wilt disease.

Regardless of the changes in C and N concentrations, C and N inputs through litterfall components were a function of the remaining basal area along the gradient of disturbance intensities. For example, C and N inputs through needle litterfall decreased with an increased disturbance intensity caused by pine wilt disease due to a considerable difference in the stand basal area in response to the removal of infected trees. Previous studies have reported that the nutrient input through litterfall tended to decrease roughly in proportion with the degree of dead or removed trees [2,25]. Carbon inputs through branches and bark litterfall were also linearly related to the disturbance intensity caused by pine wilt disease (Figure 2), although wood litter input could be affected by abiotic factors, such as storms or strong winds, rather than differences in the stand basal area [20].

Regarding our hypothesis, the results indicate that the intensive removal of pine wilt disease-infected trees could decrease the C and N inputs through litterfall. Considering that litterfall is a major source of soil organic C and N, the detected reduction in C and N inputs through litterfall could have further impacts on C and N storage in the infected forest soils [26]. It indicates that the effects of infected tree removal should be considered in future studies interpreting C and N inputs through litterfall in forests impacted by pests.

4.2. Decomposition Rates of Needle Litter and Branches

Stand basal area was not a major factor affecting the decomposition rates of needle litter in pine wilt disease disturbed stands. Although decomposition activities are sensitive to changes in soil temperature and water content, both soil environmental factors in this study were not a strong driver of needle decomposition rates. This allows us to expect that the stimulating effect of microclimatic changes on litter decomposition might be counteracted by the limitation of soil nutrients for decomposers under intensive removal of infected trees. Similarly, previous studies reported that the decomposition of needle litter was seldom affected by tree removal [1,7]. Conversely, another study reported that the decomposition rates of pine needles decreased following canopy removal because of a decrease in meso-fauna abundance due to increased light levels [27]. Such inconsistencies in the relationship between the basal area and the decomposition rates of needle litter could be due to the complex responses of abiotic and biotic factors following canopy removal [1,7].

Although the decomposition rate of branches was related to the basal area, it is not clear whether the decreased decomposition rates of branches in the severely disturbed plots could be attributed to changes in microclimatic factors such as soil temperature and water content (Figure 6), which are generally the two most influential factors for decomposer communities. It was found that soil water content tended to increase with the removal of infected trees, which is the opposite of the branch decomposition rate. This result suggests that the decomposition of branches could be affected by environmental factors other than increased temperature and water infiltration following canopy removal. For instance, a meta-analysis by Holden and Treseder [28] suggested that the incidence of tree-killing pine beetles could result in a notable decline in the soil microbial community. Given that soil nutrient availability decreased in the intensively disturbed plots (Table 2), the decline in soil microbes resulting from nutrient limitation might play a role in the lower branch decomposition rate under the intensive removal of the infected trees. Photodegradation, a process by which solar radiation breaks down organic matter components, would greatly affect the decomposition rates of wood litter through a considerable increase in solar radiation in the severely disturbed plots [29]. For instance, the activity of the decomposing microorganisms [30] may be limited to increased solar radiation with a high C/N ratio in severely disturbed plots (Figure 5d).



Figure 6. (a) Annual mean soil temperature, (b) soil water content, and (c) soil pH among basal area treatments in pine wilt disease disturbed stands (n = 3). The different letters among the basal area treatments for each year indicate significance at p < 0.05. The same color across the years denotes the same basal area treatment (disturbance intensity). Vertical bars represent standard error.

4.3. Carbon and Nitrogen Responses during Decomposition Processes of Needle Litter and Branches

The remaining C in decomposing needle litter and branches was generally lower in the slightly disturbed plots (high basal area) than in the severely disturbed plots (low basal area), even though the C concentration and remaining C generally decreased over the process of decomposition compared to those of undecomposed needles or branches. The difference in the remaining C between the disturbance intensities is more distinct for the decomposition of branches compared with the decomposition of needle litter (Figures 4e and 5e). As decomposing litters generally lose C via diverse processes [26] such as the leaching of soluble carbohydrates and respiration of labile compounds, the lower remaining C in the slightly disturbed plot compared with the severely disturbed plots could be attributed to rapid C mineralization throughout the decomposition process. In contrast to the C concentration, needle litters initially gained N due to an increasing N-rich microbial biomass [1] during the decomposition process, especially in the slightly disturbed plots. The higher N retention in decomposing needle litter in slightly disturbed plots during early incubation could be due to increased loss of C from the decomposing litter or depressed microbial activity under soil nutrient limitations in severely disturbed plots [13]. In fact, fungal mycelia, a major driver of microbial N immobilization, was rarely observed in the severely disturbed plots during the study period. However, the remaining N in decomposing branches was

not linearly related to the basal area during the two sampling times (Figure 5f). This might occur because, compared with severely disturbed plots, slightly disturbed plots showed generally higher N retention in decomposing branches via microbial immobilization. This pattern might counteract the faster branch decomposition under the slight removal of the infected trees and consequently confound the relationship between the remaining N of decomposing branches and basal area.

The overall results demonstrate that the relationship between the disturbance intensity and C and N release through decomposition was relatively unclear, given that C and N inputs through litterfall exhibited a direct relationship with basal area (disturbance intensity). This finding shows that the decrease in forest floor C storage along increasing disturbance intensities caused by pine wilt disease [12] might result from the gradual reduction in litterfall rather than alterations to the litter decomposition rate. Our results also suggest that it is necessary to consider the inconsistent effect of tree removal intensity on C and N release through decomposition rates when interpreting soil C and N dynamics under disturbed conditions after pine wilt disease.

5. Conclusions

The disturbance intensity of pine wilt disease greatly altered the quality and quantity of C and N in litterfall components and the decomposition processes of needle litter and branches. Removal of infected trees reduces the C and N inputs through litterfall components and the decomposition of branches. These effects were attributed to modifications in microclimatic conditions and a reduction in soil nutrient availability by removing trees killed by pine wilt disease. Thus, C and N loss of pine wilt disease stands may have contributed to long-term declines in forest productivity. The results indicate that it is critical to understand the impacts on the C and N status at various degrees of tree removal because of the possible significant changes to the soil nutrient cycle through litterfall and decomposition processes in pine wilt disease stands.

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References

- 1. Berg, B.; Laskowski, R. Litter decomposition; A guide to carbon and nutrient turnover. *Adv. Ecol. Res.* 2006, *38*, 20–71.
- 2. Morehouse, K.; Johns, T.; Kaye, J.; Kaye, M. Carbon and nitrogen cycling immediately following bark beetle outbreaks in southwestern ponderosa pine forests. *For. Ecol. Manag.* **2008**, *255*, 2698–2708. [CrossRef]
- 3. Hicke, J.A.; Allen, D.C.; Desai, A.R.; Dietze, M.C.; Hall, R.J.; Hogg, E.H.; Kashian, D.M.; More, D.; Raffa, K.F.; Sturrock, R.N.; et al. Effects of biotic disturbances on forest carbon cycling in the United States and Canada. *Glob. Chang. Biol.* **2012**, *18*, 7–34. [CrossRef]
- Gao, R.; Shi, J.; Huang, R.; Wang, Z.; Luo, Y. Effects of pine wilt disease invasion on soil properties and Masson pine forest communities in the three Gorges reservoir region, China. *Ecol. Evol.* 2015, *5*, 1702–1716. [CrossRef] [PubMed]
- 5. Kim, C.; Jeong, J.; Cho, H.S.; Lee, K.S.; Park, N.C. Carbon and nitrogen status in litterfall of a red pine stand with varying degrees of damage from pine wilt disease. *J. Ecol. Field Biol.* **2011**, *34*, 215–222. [CrossRef]
- 6. Bates, J.D.; Svejcar, T.S.; Miller, R.F. Litter decomposition in cut and uncut western junifer woodlands. *J. Arid Environ.* **2007**, *70*, 223–236. [CrossRef]

- Kim, C.; Sharik, T.L.; Jurgensen, M.F. Canopy cover effects on mass loss, and nitrogen and phosphorus dynamics from decomposing litter in oak and pine stands in northern Lower Michigan. *For. Ecol. Manag.* 1996, *80*, 13–20. [CrossRef]
- 8. Kim, C.; Son, Y.; Lee, W.K.; Jeong, J.; Noh, N. Influences of forest tending works on carbon distribution and cycling in a *Pinus densiflora* S. et Z. stand in Korea. *For. Ecol. Manag.* **2009**, 257, 1420–1426. [CrossRef]
- Lado-Monserrat, L.; Lidón, A.; Bautista, I. Erratum to: Litterfall, litter decomposition and associated nutrient fluxes in *Pinus halepensis*: influence of tree removal intensity in a Mediterranean forest. *Eur. J. Forest Res.* 2016, 135, 203214. [CrossRef]
- Pérez, G.; Díer, J.J.; Ibeas, F.; Pajares, J.A. Assessing pine wilt disease risk under a climate change scenario in northwestern Spain. In *Managing Forest Ecosystem: The Challenge of Climate Change*; Bravo, F., LeMay, V., Jandl, R., Gadow, K., Eds.; Springer: New York, NY, USA, 2009; pp. 269–282. [CrossRef]
- Ikegami, M.; Jenkins, T.A.R. Estimate global risks of a forest disease under current and future climate using species distribution model and simple thermal model-pine wilt disease as a model case. *For. Ecol. Manag.* 2018, 409, 343–352. [CrossRef]
- 12. Jeong, J.; Kim, C.; Lee, K.S.; Bolan, N.; Naidu, R. Carbon storage and soil CO₂ efflux rats at varying degrees of damage from pine wit disease in red pine stands. *Sci. Total Environ.* **2013**, *465*, 273–278. [CrossRef] [PubMed]
- 13. Mabuhay, J.A.; Nakagoshi, N. Response of soil microbial communities to changes in a forest ecosystem brought about by pine wilt disease. *Landscape Ecol. Eng.* **2012**, *8*, 189–196. [CrossRef]
- 14. Kwon, T.S.; Shin, J.H.; Lim, J.H.; Kim, Y.K.; Lee, E.J. Management of pine wilt disease in Korea through preventative silvicultural control. *For. Ecol. Manag.* **2011**, *261*, 562–569. [CrossRef]
- 15. Kim, C.; Jang, K.-S.; Kim, J.-B.; Byun, J.-K.; Lee, C.-H.; Jeon, K.-S. Relationship between soil properties and incidence of pine wilt disease at stand level. *Landscape Ecol. Eng.* **2010**, *6*, 119–124. [CrossRef]
- 16. Jeon, K.S.; Kim, C.S.; Park, N.C.; Hur, T.C.; Hong, S.C. Effects on control of pine wilt disease (*Bursaphelenchus xylophilus*) by thinning methods in red pine (*Pinus densiflora*) forest. *J. Korean For. Soc.* **2011**, *100*, 165–171.
- 17. Hurlbert, S.H. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* **1984**, *54*, 187–211. [CrossRef]
- Kalra, Y.P.; Maynard, D.G. Methods Manual for Forest Soil and Plant Analysis; Northwest Region, Information Report. NOR-X-319; Northern Forestry Centre: Edmonton, AB, Canada, 1991; p. 115.
- 19. SAS Institute Inc. SAS/STAT Statistical Software; Version 9.1; SAS Publishing: Cary, NC, USA, 2003.
- 20. Kim, C. Basal area effects on a short-term nutrient status of litter fall and needle litter decomposition in a *Pinus densiflora* stands. *J. Ecol. Environ.* **2016**, *39*, 51–60. [CrossRef]
- 21. Bueis, T.; Bravo, F.; Pando, V.; Turrión, M.B. Local basal area affects needle litterfall, nutrient concentration, and nutrient release during decomposition in *Pinus halepensis* Mill. plantations in Spain. *Ann. For. Sci.* **2018**, 75, 21. [CrossRef]
- 22. Hansen, K.; Vesterdal, L.; Schmidt, I.K.; Gundersen, P.; Sevel, L.; Bastrup-Birk, A.; Pedersen, L.B.; Bille-Hansen, J. Litterfall and nutrient return in five tree species in a common garden experiment. *For. Ecol. Manag.* **2009**, 257, 2133–2144. [CrossRef]
- 23. Livesley, S.J.; Ossola, A.; Threlfall, C.G.; Hahs, A.K.; Williams, N.S.G. Soil carbon and carbon/nitrogen ratio change under tree canopy, tall grass, and turf grass areas of urban green space. *J. Environ. Qual.* **2015**, 545, 215–223. [CrossRef]
- 24. Peri, P.L.; Gargalione, V.; Pastur, G.M.; Lencinas, M.V. Carbon accumulation along a stand development sequence of Nothofagus Antarctica forests across a gradient in site quality in Southern Patagonia. *For. Ecol. Manag.* **2010**, *260*, 229–237. [CrossRef]
- 25. Lorenz, K.; Lal, R. Carbon Sequestration in Forest Ecosystems; Springer: New York, NY, USA, 2010; pp. 103–158.
- Ge, P.; Da, L.J.; Wang, W.B.; Xu, X.N. Seasonal dynamics of dissolved organic carbon, nitrogen and other nutrients in soil of *Pinus massoniana* stands after pine wilt disease disturbance. *J. Soil Sci. Plant Nutri.* 2014, 14, 75–87. [CrossRef]
- 27. Blanco, J.A.; Imbert, J.B.; Castillo, F.J. Thinning affects *Pinus sylvestris* needle decomposition rates and chemistry differently depending on site conditions. *Biogeochemistry* **2011**, *106*, 397–414. [CrossRef]
- Holden, S.R.; Treseder, K.K. A meta-analysis of soil microbial biomass responses to forest disturbances. *Front. Microbiol.* 2013, 4, 163. [CrossRef] [PubMed]

- 29. Witkamp, M. Decomposition of leaf litter in relation to environment, microflora, and microbial respiration. *Ecology* **1966**, 47, 194–201. [CrossRef]
- Angst, Š.; Cajthaml, T.; Angst, G.; Šimàckovà, H.; Brus, J.; Frouz, J. Retention of dead standing plant biomass (marcescence) increases subsequent litter decomposition in the soil organic layer. *Plant Soil* 2017, 418, 571–579. [CrossRef]



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