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Fine-root vitality in a Norway spruce stand subjected to various nutrient supplies

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Abstract

The vitality of fine roots in a Norway spruce stand subjected to application of ammonium sulphate (NS), wood ash (A) and nitrogen-free fertilizer (V) respectively, was investigated using i) vitality classification of fine roots based on morphological characteristics and ii) the triphenyl tetrazolium chloride (TTC) method of estimating dehydrogenase activity.

Although the NS-treated areas showed a 30% increase in above-ground production in response to the NS-application, the vitality of the fine-root system in the humus layer appeared to be in a state of deterioration, as indicated by a decrease in fine-root biomass, an increased amount of dead fine (0–1 mm) and small (1–2 mm) roots, a decreased specific root length (SRL = fine root length/fine root dry weight) and an increased dehydrogenase activity. The impact of the the A and V treatments was reflected in a decrease in fine-root biomass and an increase in SRL. The results make it clear that in order to study the vitality of forest trees, both fine-root studies and studies of above-ground tree parts are necessary.

Introduction

During the last 20–30 years there has occurred an increase in soil acidification and a leakage of mineral nutrients from forest soils in Southern Sweden (Falkengren-Grerup, 1987; Tamm and Hallbäcken, 1988). So far, forest tree productivity has not been affected, and many forest stands show a growth response to the increased input of nitrogen, even in the most heavily nitrogen-loaded areas (Nilsson and Wiklund, 1992). However, in areas of Germany with more extensive acidification and nutrient leakage, a decline in tree vitality has been reported (Hüttermann, 1985; Schulze, 1989). The judgement is based mainly upon studies of needle damage and needle loss, although more and more attention is now being drawn to the vitality of the tree root systems and to the fine roots which are the structures that are most essential in nutrient and water uptake. Root damage is often observed as a decline in the amount of living fine roots and an increase in the amount of dead and coarse roots (Persson, 1993).

A problem in this context is the definition and quantification of root vitality. Different approaches have counted root tips, with or without taking mycorrhizal infection into consideration (Ahlström et al., 1988; Blaschke and Weiss, 1990), estimated mineral nutrient contents (Eichhorn et al., 1988; Kimmins and Hawkes, 1978; Yin et al., 1991), and analyzed root ingrowth into root-free soil cores (Persson and Ahlström, 1991). Triphenyl tetrazolium chloride (TTC) is a chemical which, when added to a tissue, is reduced by enzymes, mainly the group of dehydrogenases (Steponkus, 1971). The method has been used for the study of the vitality of different tissues, e.g. in the detection of frost injuries in plant seedlings (Lassheiki et al., 1991; Lindström and Nyström, 1987), and in distinguishing between living and dead roots in mature trees (Joslin and Henderson, 1984). The degree of suberization is also a feature that is related to vitality. In tree roots, often only the most recently formed roots are unsuberized. For many nutrients the uptake rates are higher in the younger and unsuberized portions of the roots (Bledsoe and Atkinson, 1991; Jensén and Pettersson, 1980). However, considering the extreme-

ly large amounts of suberized tree roots, it has been argued that suberized roots may be the major route by which nutrients are taken up (cf. Bowen, 1984).

The aim of the present study was to investigate the vitality of fine roots subjected to high nitrogen and sulphur loads, nitrogen-free fertilizer and wood ash, respectively. The two different approaches used were: i) a vitality classification of fine roots based on morphological characteristics, together with an estimation of other root variables; and ii) a quantification of dehydrogenase activity in fine roots as measured by the TTC-reduction method.

Materials and methods

Site description

The experimental site Skogaby is a 28-year-old Norway spruce (*Picea abies* (L.) Karst) stand in SW Sweden (lat. 56°33', long. 13°13', alt. 95–115 m a. s. l.). The soil is a sandy till with a poorly developed podzol. The pH of the soil lies between 3.9 and 4.5, and it is poor in basic cations and rich in aluminium. The experimental layout was a randomized block design with four replicates. A more detailed description of the experimental site has been made by Nilsson and Wiklund (1992). The following treatments were studied:

- Control (C);
- Ammonium sulphate (NS), 100 kg N + 114 kg S ha^{-1} yr^{-1};
- Wood ash (A), 4000 kg ha^{-1} of granulated wood ash as a single dose;
- Nitrogen-free fertilizer (V), 100 kg ha^{-1} of Skogvital, a commercial fertilizer, applied once annually during a two year period;

The treatments commenced in 1988, with the exception of the ash application which was started in 1989. A list of the nutrient composition of each treatment is shown in Table 1.

Soil sampling was carried out in September 1992. A steel corer with an inner diameter of 7.0 cm was used for the sampling of the humus layer and a core auger with an inner diameter of 4.5 cm was used for that of the mineral soil. Eight samples were taken in the humus layer and four samples were taken in the mineral soil to a depth of about 30 cm, in each of the four replicated areas in each treatment.

Immediately after transportation to the laboratory, half of the samples from the humus layer were kept at +4°C and were used in the TTC-method. The remaining samples were stored in a freezer at -4°C until sorting could take place.

The TTC-method

The TTC-method used in the present investigation has been described by Lindström and Nyström (1987). Spruce fine roots were picked out and stored in tap water overnight. The roots where then cut into small pieces, 1–2 mm long, and 200 mg were weighed and put into test tubes. Six ml of 0.6% (w/v) TTC in 0.06 *M* Na_2HPO_4 - KH_2PO_4 and 0.05% (v/v) wetting agent (Tween 20, Kebo AB, Sweden) were added and the samples were vacuum-infiltered for 15 min. before incubation for 20 hours at 30°C. The samples were extracted with 95% (v/v) ethanol in a waterbath kept at 80°C for 15 min. The absorbance at 520 nm was recorded.

Fine-root processing

After thawing the soil samples, spruce roots were picked out and separated into the different diameter classes, viz. 0–1 mm, 1–2 mm and 2–5 mm. Fine roots of 0–1 mm were in turn separated into 3 vitality classes, based on the following morphological characteristics:

- Vitality class 1: The roots were more or less suberized, well branched, with several white, turgid root tips. Few root tips were darkened and/or fell off. The stele was white and elastic.
- Vitality class 2: The roots were darkened. White root tips were often few in number. The stele was still elastic and light to slightly brown.
- Vitality class 3: The roots in this class are normally referred to as dead. The stele was brownish and easily broken. No elasticity remained. The main portion of the root tips were blackened and fell off.

Roots 1–2 mm and 2–5 mm in diameter were sorted into living and dead, according to conventional characteristics (Vogt and Persson, 1991). Root length was measured using air-dried samples and a Comair root length scanner for fine roots 0-1 mm in diameter and a ruler in the case of coarser roots. After drying for 48 hours at 70°C, the roots were weighed to the nearest mg.

Statistics

Significant differences between treatments were tested by the ANOVA (SAS GLM) analysis of variance and the Student's t-test ($p<0.05$) (Ray, 1982).

Table 1. Macro nutrients supplied in the different treatment areas at Skogaby. A = wood ash; NS = ammonium sulphate; V = nitrogen-free fertilizer ("Skog-vital")

Treatment	Macro nutrient						Treatment period
	N	P	K	Ca	Mg	S	
A	0	25	129	448	47	4	Totals as a single dose in 1989
NS	100	0	0	0	0	114	Annually
V	0	48	43	218	46	75	Totals during 1988–89

Results

Vitality classes

The amount of fine roots (0–1 mm in diameter) belonging to class 1 and found in the humus layer was significantly higher in the control areas, when compared with all the other treatment areas (Fig. 1). Classes 2 and 3 fine roots differed from the control only in the NS-treatment areas, with a lower amount of class 2 and a higher amount of class 3 (Fig. 1). The total amount of living fine roots (class 1 and 2) was highest in the control areas, while the treated areas showed significantly lower amounts (Table 2). The amount of fine roots belonging to separate vitality classes expressed as a percentage of the total amount of fine roots (all classes together) differed from the control areas as regard classes 1 and 2 in the ash treatment areas, with a lower percentage of class 1 and higher one of class 2 respectively (Table 2). The NS-treatment areas had a significantly higher percentage of class 3 (dead fine roots) compared with control areas (Table 2). The other treatments showed no differences.

Living and dead small roots 1–2 mm in diameter differed significantly from the control areas only in the NS-treatment areas, with a lower amount of living small roots and a higher amount of dead small roots (Table 2). Roots 2–5 mm in diameter occurred in similar quantities in the different treatment areas (Results are not reported).

In the mineral soil there were few differences; those occurred mainly in the upper 5 cm of the soil. The amount of dead fine roots (class 3) was significantly higher in the ash and the NS-treatment areas when compared with the control. The percentage of class 2 fine roots was significantly higher in the upper 5 cm of the soil in the control areas compared with all the other treated areas. Generally, a very small portion of the fine roots in the mineral soil were considered to belong to class 1. No differences in the deeper horizons were observed (Table 2).

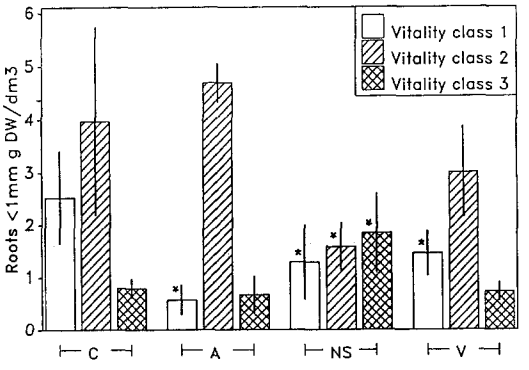

Fig. 1. Amount of fine roots 0–1 mm in diameter, separated into three vitality classes, in the humus layer of different treatment areas. Mean values ± SD. Number of replicates is 4, each based on 4 samples. C = control; A = wood ash; N = ammonium sulphate; V = nitrogen-free fertilizer. Significant differences (Student's t-test. $p<0.05$) from the control are indicated (*).

The specific root length (SRL) was significantly higher in both the A- and the V-treatments in the case of class 1 roots compared with that in control areas. The NS-treatment showed a lower SRL for class 2 roots (Table 3).

Dehydrogenase activity

The dehydrogenase (DH) activity in fine roots, as measured by the TTC-reduction method, changed significantly in both the NS- and V-treatments when compared with that in control areas (Fig. 2). Roots in the V-treatment areas decreased their DH-activity, while roots in the NS-treatment areas increased theirs.

Table 2. Amount of roots (g DW dm^{-3}) in different root fractions (0–1 mm and 1–2 mm in diameter) in the soil profile of different treatment areas. Living roots 0–1mm in diameter correspond to vitality classes 1 and 2. Number of replicates is 4, each based on 4 samples. Significant differences (Student's t-test, $p<0.05$) compared with controls are indicated (*). C = control; A = wood ash; NS = ammonium sulphate; V = nitrogen-free fertilizer. Mean values ± SD

Soil	Living roots		Dead roots		Percentage of class		
Depth (cm)/ Treatments	0–1mm	1–2mm	0–1mm	1–2mm	1	2	3
LFH							
C	6.5 ± 0.9	2.7 ± 0.3	0.8 ± 0.2	0.7 ± 0.3	35 ± 14	51 ± 18	14 ± 6
A	5.3 ± 0.5*	2.6 ± 1.0	0.7 ± 0.3	0.4 ± 0.3	9 ± 4*	79 ± 9*	12 ± 5
NS	2.9 ± 0.4*	1.8 ± 0.4*	1.8 ± 0.8*	1.3 ± 0.5*	28 ± 17	33 ± 7	40 ± 13*
V	4.5 ± 0.9*	2.0 ± 0.6	0.7 ± 0.2	0.4 ± 0.3	29 ± 6	57 ± 6	15 ± 3
Mineral soil 0–5(5 cm)							
C	1.0 ± 0.3	0.6 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	2 ± 3	74 ± 7	23 ± 5
A	0.7 ± 0.3*	0.4 ± 0.2	0.6 ± 0.3*	0.1 ± 0.1	0 ± 0	55 ± 22*	45 ± 22
NS	1.0 ± 0.2	0.7 ± 0.2	0.7 ± 0.0*	0.0 ± 0.0	2 ± 4	54 ± 11*	44 ± 7
V	0.7 ± 0.2	0.6 ± 0.1	0.5 ± 0.2	0.1 ± 0.2	4 ± 4	52 ± 3*	44 ± 8
Mineral soil 5–15(10 cm)							
C	0.5 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	0.1 ± 0.1	1 ± 1	58 ± 13	41 ± 12
A	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.0	0.1 ± 0.1	0 ± 0	41 ± 25	59 ± 25
NS	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.0	0 ± 0	50 ± 14	50 ± 14
V	0.3 ± 0.0	0.3 ± 0.2	0.3 ± 0.0	0.1 ± 0.0	0 ± 0	43 ± 9	57 ± 9
Mineral soil <15							
C	0.3 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0 ± 0	41 ± 4	59 ± 4
A	0.2 ± 0.3	0.2 ± 0.3	0.2 ± 0.1	0.3 ± 0.1*	0 ± 0	30 ± 17	70 ± 17
NS	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.0	0.1 ± 0.1	0 ± 0	50 ± 7	50 ± 7
V	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0 ± 0	24 ± 22	76 ± 22

Table 3. Specific root length (SRL=fine root length/fine root dry weight, m/g) of roots in vitality class 1 (SRL1) and 2 (SRL2) respectively. Mean values ± SD. Number of replicates is 4, each based on 4 samples. Significant differences (Student's t-test, $p<0.05$) from the control are indicated (*). C = control; A = wood ash; NS = ammonium sulphate; V = nitrogen-free fertilizer

		Treatment			
		C	A	NS	V
Humus layer	SRL1	16 ± 3	20 ± 1*	15 ± 2	19 ± 2*
	SRL2	15 ± 1	15 ± 0	13 ± 1*	15 ± 1

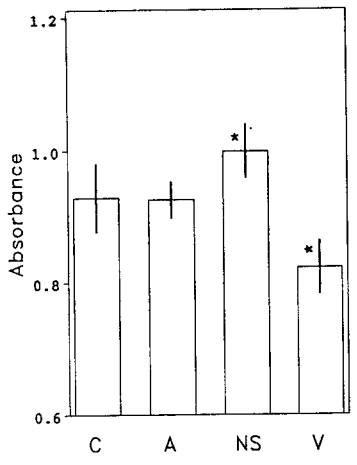

Fig. 2. Dehydrogenase activity measured by the TTC-reduction method. Absorbances are given as mean values ± SD. Number of replicates is 4, each based on 4 samples. C=control; A = wood ash; N = ammonium sulphate; V = nitrogen-free fertilizer. Significant differences (Student's t-test, $p<0.05$) from the control are indicated (*).

Discussion

Although the NS-treated areas demonstrated an increase in stem production of more than 30% compared with that in the controls (Nilsson and Wiklund, 1992), the fine-root system in the humus layer showed symptoms of stress, as indicated by a deterioration in fine-root vitality and a decrease in rooting density. An increase in dead fine (0–1 mm, class 3) and small (1–2 mm) roots was also observed. Earlier root samplings carried out in the same area during 1988–1990 (Majdi and Persson, 1995) indicate increasing amounts of dead fine roots in the humus layer of the NS-treatment areas when compared with controls in 1990.

The SRL increased, i.e. the roots became thicker, which is in agreement with the results obtained by Olsthoorn et al. (1991) who found a decrease in the SRL of Douglas-fir seedlings treated with high levels of ammonium sulphate. A reduction in mycorrhizal infection was also indicated in the NS-treatment through a strong reduction in the number of mycorrhizal fruit bodies (Nilsson, pers. comm.).

The implications of all these factors are a deterioration in root function and nutrient uptake, which, in the NS-treatment, are reflected in a low nitrogen to phosphorus ratio (7.6%) in current needles (Nilsson and Wiklund, 1992). The ability of the tree to maintain a satisfactory above-ground production in the long term could therefore be expected to be impaired in the NS-treatment areas.

The increase in the DH-activity of fine roots in the NS-treatment can be compared with the general increase in enzymatic activity found in diseased spruce trees subjected to environmental pollution (Gerant et al., 1987). Increased DH-activity with increasing NH_4-N supplements has also been indicated by Clemensson-Lindell (1994). However, the use of DH-activity as a general indicator of stress may lead to a simplification of the results, since the DH-enzymes are not coupled to any specific function.

The most striking result is how tree vitality assessments may point in varying directions, depending on which part of the tree is studied. Obviously, it is not enough to estimate tree vitality solely from above-ground productivity. Possibly, more functional parameters, such as nutrient uptake and specific enzyme activities in fine roots, may provide more information.

The purpose of the other treatments, viz. the wood ash application (A) and the nitrogen-free fertilization (V), was to increase the vitality of the trees and their root systems. The increased SRL found in the most vital fine roots in both treatments is thought to imply an improved nutrient uptake per given root weight (Bledsoe and Atkinson, 1991). However, since the biomass of fine roots decreased in the A- and the V-treatments, the total benefit achieved as regards nutrient uptake is not clear. The reason for this morphological effect in fine roots may possibly be related to alterations in pH or to the high calcium content in both treatments. A similar tendency towards higher SRL was found in areas subjected to different types and levels of lime application (Clemensson-Lindell and Persson, 1993).

Conclusions

- The vitality of the fine-root system in the NS-treated areas appeared to be in a state of deterioration, although the trees displayed a 30% increase in above-ground production in response to nitrogen and sulphur application.
- The effects on fine roots were observed mainly in the humus layer.
- The impact of nitrogen-free fertilizer and wood ash on fine-root vitality appeared contradictory, inasmuch as it decreased fine-root biomass on the one hand and increased SRL, which is thought to improve nutrient uptake on the other.

- The results make it clear that studies of roots and above-ground tree parts are equally important when attempting to assess the vitality of forest trees as a whole.

Acknowledgements

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