Applied Soil Ecology 168 (2021) 104101

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Short Communication

Ecology, biology and enzymatic activity of the rhizosphere planted with *Larix decidua* seedlings after addition of vermicompost

Sebastian Wojciech Przemieniecki [a,*], Andrzej Skwiercz [b], Marta Damszel [a], Arkadiusz Telesiński [c], Anita Zapałowska [d], Zbigniew Sierota [e], Anna Gorczyca [f]

[a] University of Warmia and Mazury in Olsztyn, Department of Entomology, Phytopathology and Molecular Diagnostics, Prawocheńskiego 17, 10-720 Olsztyn, Poland
[b] Research Institute of Horticulture in Skierniewice, Department of Pests Management, Pomologiczna 18, 96-100 Skierniewice, Poland
[c] West Pomeranian University of Technology Szczecin, Department of Bioengineering, Słowackiego 17, 71-434 Szczecin, Poland
[d] University of Rzeszow, College of Natural Sciences, Institute of Agricultural Sciences, Land Management and Environmental Protection, Ćwiklińskiej 1a, 35-601 Rzeszow, Poland
[e] University of Warmia and Mazury in Olsztyn, Department of Forestry and Forest Ecology, Pl. Łódzki 2, 10-727 Olsztyn, Poland
[f] University of Agriculture in Krakow, Department of Microbiology and Biomonitoring, Mickiewicza 21, 31-120 Krakow, Poland

ARTICLE INFO

Keywords:
Forest nurseries
Vermicompost
Mycorrhization
Nematode community
Rhizosphere microbiota

ABSTRACT

The aim of this study was to determine the influence of *Eisenia fetida* vermicompost (VC) added to forest nursery soil. The chemical reaction in the soil and selected microorganisms, such as bacteria, fungi and nematodes, were assessed. Larch seedlings were cultivated for 2 years in pots with nursery soil as a control and VC-amended soil in two doses: 10% (V10) and 20% (V20) *v*/v. One important observation was the connection between the addition of VC and the improvement of the plant's biometry and a much higher level of mycorrhization with increasing *Suillus* sp. Vermicompost modified the nematode community by reducing plant parasitic nematodes, and significantly increasing bacterial and fungivorous, as well as predatory nematodes. Actinomycetes bacteria, which were present in a proportion of 2.2% in the control soil, increased to 10.5% in V10, and to 5.4% in V20. *Bacillus* spp. increased along with the amount of VC from <0.01% in the control to 2.2% in V10 and to 2.7% in V20. The C:P ratio correlated negatively while the C:K ratio and N-NH_4 concentration correlated positively with the abundance of *Bacillus* spp. Toxigenic *Penicillium* spp. and *Fusarium* spp. were suppressed. The stability of VC-amended soil system with respect to enzymatic activity was rather high, except for o-diphenol oxidase which was strongly reinforced by the VC. The enzyme activity values for all enzymes were correlated with an improvement in the values of the microbiological, biometric and nematode parameters and increased mycorrhization. One favorable modification, which was manifested in better seedling growth, was the effect of VC on the development of *Suillus*-morphotype mycorrhizal fungi, fungal feeder nematodes and *Bacillus* spp. Vermicompost may therefore be recommended in practice as a method of sustainable management for soil protection in larch forest nurseries.

1. Introduction

An interaction between nematodes, microbiota and earthworms producing vermicompost (VC) in plant crops has been clearly described (Edwards, 2004; Thakuria et al., 2010; Niu et al., 2019). By promoting beneficial microorganisms, VC stimulates the growth and development of plants both directly (production of hormones and enzymes regulating growth) and indirectly (control of pathogens and pests). Vermicompost also contributes to the sustainable development of agriculture through the safe management of waste. Vermicompost is a low-cost and eco-biotechnological product that improves soil properties. It is also a fertilizer and a plant growth stimulator that does not adversely affect the natural environment as mineral fertilization does (Pathma and Sakthivel, 2012; Blouin et al., 2013; Van Groenigen et al., 2014; Song et al., 2015).

The presence of beneficial microbiota and nematodes in the soil which is stimulated by VC is very desirable to enrich the soil web with various trophic links (Medina-Sauza et al., 2019). Many research studies have been conducted on this topic regarding agricultural and horticultural crops, but little research has been done concerning forest crops. It is a topic that is of growing importance, especially at a time when intensive efforts are being made to encourage forestation due to climate

* Corresponding author.
E-mail address: sebastian.przemieniecki@uwm.edu.pl (S.W. Przemieniecki).

https://doi.org/10.1016/j.apsoil.2021.104101
Received 20 September 2020; Received in revised form 22 April 2021; Accepted 31 May 2021
Available online 17 June 2021

change (UNFCCC, 2015).

The substrates used in forest nurseries are mostly peat and bark. These substrates meet the requirements with regard to their physical properties but, in their fresh state, are free of microbiota. Peat and bark have a very low content in basic nutrients and fertilization is therefore required. Fertilization is usually carried out by the application of solid mineral fertilizers or sprinkler system. In this way, the mineral fertilizers are dissolved in irrigation water and delivered to the seedlings. All the components involved in this technology (peat, bark, mineral fertilizers) are expensive and not sustainable (Pascual et al., 2018). Vermicompost is one of the organic fertilizers that is recommended for use in the sustainable production of nursery substrates. The first meta-analysis of literature data conducted indicates that VC has a significant positive effect on the growth of agricultural plants (Blouin et al., 2019), resulting in a growing inclination to conduct research in other areas of plant cultivation. Utilization of VC in forest nurseries is a first step in the improvement of the sustainable technology in forest management. The question is how the above-mentioned VC-induced interactions take place in the substrates in nurseries. So far, the few studies done into the use of VC in forest nurseries have focused on assessment of the biometry of tree seedlings. The stimulating effect of low doses of VC in nursery substrates on the development of roots and stems of *Pinus nigra* (Atik et al., 2015), *P. pinaster* (Lazcano et al., 2010a, 2010b), *P. gerardiana* (Raj Kumar et al., 2016), *Eucalyptus* (Asghari and Rafiei, 2013), *Betula platyphylla*, *Larix kaempferi* and *Chamaecyparis obtusa* (Dao et al., 2020) has been proven. In the studies by Dao et al. (2020) *L. kaempferi*, regardless of the methods of placement used for application of VC in pots, a significant improvement was achieved in stem growth and biomass, as well as in root collar diameter and higher foliar N concentration compared to the control. Analyzing 68 papers, presenting a total of 665 relevant research results, Blouin et al. (2019) pointed to the lack of data in the literature on the effect of VC on the root development of trees and the very scarce amount of data on the shoot–root ratio. None of the studies on the impact of VC on trees assessed changes in the rhizosphere microbiome of seedlings, including larch seedlings, resulting from the use of those organic fertilizers.

The gaps in knowledge identified above gave us grounds to conduct research to examine the rhizosphere microbiome of European larch (*Larix decidua* Mill.) seedlings growing in nursery soil with the addition of VC obtained by using *Eisenia fetida*. European larch is one of the basic forest-forming species in temperate climate zones (according to the Köppen–Geiger climate classification) with moderate mean monthly temperatures above 10 °C in the warmest months and above −3 °C in colder months. This species is known to be a very long-lived, fast-growing coniferous tree that is able to tolerate cold temperatures (down to as low as −30 °C) and dry soils (Shuman et al., 2011; Da Ronch et al., 2016). However, in recent years, it has been affected by various diseases and pests, and therefore the production of planting material has increased in many commercial nurseries (Kowalczyk and Neyko, 2011; Hirano et al., 2017). Our hypothesis was that the presence of VC would increase the overall activity of the soil, which would, in turn, result in a decrease in the parasitic nematode population, positive changes in the mycorrhizal status of roots, and finally, an increase in the biometric parameters of seedlings and their high vitality. European larch as a species is a valuable element of forest ecosystems from an ecological perspective (Migliavacca et al., 2008), and is also of economic value, as its wood is widely used for carpentry, furniture, and as pulp for paper (Da Ronch et al., 2016), the results presented here may be useful for the cultivation of larch under controlled conditions. Benefits of the use of VC in forest nurseries include sustainable utilization of waste, a reduction in the costs of fertilizers and substrates used and obtaining larch seedlings of good quality.

2. Materials and methods

One-year-old L. *decidua* seedlings (healthy, about 30 cm high) were obtained from the Domatowo Forest Nursery (Wejherowo Forest District, Pomeranian Voivodeship, Poland). *Eisenia fetida* VC from the processing of typical fresh household waste in the form of grass, fruit and vegetable waste by a process of 6 months of continuous decomposition in a concrete composter was added to nursery soil (classified as Eutrophic Cambisols, with loam texture) in amounts of 10% *v*/v (V10) and 20% v/v (V20). The control was nursery soil. Seedlings were planted in 10 l plastic pots with prepared substrates in ten replications (60 seedlings altogether). Good tree nursery practice was followed as recommended for the pot cultivation of conifers, without fertilization and protection treatments (Klimek et al., 2011, 2013). Weeds were regularly removed. The same amount of soil irrigation was used for each of the individual objects. All analyses were performed after 2 years of field vegetation of seedlings in pots.

2.1. Biometrics of seedlings and mycorrhiza assessment

Measurements of the larch seedlings were taken to determine the length of the longest root, the height above ground, and the diameter at 5 cm above the ground. Root/stem ratio was calculated by dividing root and stem length. The integrated seedling quality index based on morphological features was computed as well (Cannell and Willett, 1976; Dickson et al., 1960). The mycorrhiza assessment was performed on clean roots of seedlings of about 2 mm in diameter cut into sections and conserved in water. Photos of 1 cm of the root sections were analyzed for the length of fine roots, number of branches (ramification), and number of autotrophic and mycorrhizal tips. Morphotypes were recognized macroscopically according to Agerer (2006).

2.2. Nematoda recovery and identification

A centrifugation method was used to assess nematode population density in the rhizosphere. After centrifugation, the nematodes in samples (100 cm^3) were killed by the addition of 6% of hot formaldehyde, processed by the rapid glycerine method (Seinhorst, 1962) to obtain the permanent slides. Microscopic methods were used to assign nematoda to genus (Skwiercz et al., 2018).

2.3. Qualitative PCR of microorganisms

Soil samples (100 g from pot) for the isolation of genetic material were passed through a 2-mm sieve and ground in a mortar. 100 mg of the prepared samples were taken for DNA isolation, which was performed using a Soil DNA Purification Kit (EURx, Poland). A list of the PCR setup used in the experiment is provided in the supplementary data (Table S1). The count of Actinomycetes was determined with the dilution method using Actinomycete Isolation Agar (Sigma Aldrich, Germany). Testing was also performed to detect functional genes of Pseudomonadaceae, Actinomycetes and *Bacillus* spp. responsible for the production of antibiotics effective against phytopathogens. Details are provided in the supplementary data (Table S1). All reaction products were detected by gel electrophoresis (UVP GelDoc-it, UVP, LLC, Canada).

2.4. Chemical and enzymatic analysis of soil samples

Chemical analyses of rhizosphere samples were performed separately according to European Union Standards (PN-ISO 10390:1997, PN-R-04023:1996, PN-R-04022:1996 + Azl:2002, PN/G-04523 PB-52, ed. 3/01.06.2015) and the analytics handbook (Table S2). Samples were analyzed for the activity of enzymes such as: dehydrogenases – DHA (Casida et al., 1964), *o*-diphenol oxidase – oDPO (Perucci et al., 2000), and β-glucosidase – GLU (Eivazi and Tabatabai, 1988), acid – ACP and alkaline phosphatases – ALP (Tabatabai and Bremner, 1969), and inorganic pyrophosphatase – IPP (Dick and Tabatabai, 1978), urease – URE (Kandeler and Gerber, 1988), nitrate reductase – NR (Abdelmagid and

Tabatabai, 1987), and proteases – PROT (Ladd and Butler, 1972). The VC Effect Index (IF_V) was calculated using the method described by Kaczyńska et al. (2015). The Resistance Index (RS) was calculated according to the method described by Orwin and Wardle (2004).

2.5. Statistical analysis

The Kruskal-Wallis test was used in calculations. For biometric tests, ANOVA with the Levene, Neuman-Keuls, Kruskal-Wallis and HSD Tukey tests was used (Statistica 13.1, StatSoft). Simpson's index domination, Shannon's index of diversity, and Pielou's index of evenness, and the domination class, were calculated as in PAST 4. The Principal Component Analysis was calculated in XLSTAT (Addinsoft).

3. Results and discussion

3.1. Biometrics of seedlings

Larch seedlings grown in the nursery in VC-amended soil were characterized by better biometric parameters compared to the control, especially in the case of the V20 variant (Table 1). The integrated quality index of seedlings in both VC variants was 3–6-times greater than in the control soil. The increase in biometrics parameters reflects the nutritional status of plants, because there are significant relationships between the proportion of fine roots and biomass and the condition of needles (Danjon and Reubens, 2008; Meng et al., 2018). In the present study, the number of fine roots increased along with an increase in the dose of VC and it was significantly greater with the higher dose of VC (7 pcs) compared to the control where it was 4 pcs per 1 mm of roots. According to a meta-analysis of the effect of VC on plant growth (Blouin et al., 2019), VC has a positive effect on plants in various proportions, but doses of over 20%, up to a maximum of 60%, are usually considered to be optimal in agricultural crops. Our research showed that low doses of VC (10, 20%) are effective in substrates for forest nurseries cultivating larch seedlings, and the dose of 20% shows highly positive effects compared to the control. A meta-analysis conducted by Blouin et al. (2019) showed that higher proportions of VC (40–60%) led to a lower positive effect, or, at even higher proportions (60–80% and 80–100%), to the complete disappearance of the significant positive effect on root biomass. In our research, we have shown that the applied doses of VC have a positive effect on the elongation of larch roots and, as a result, lead to a significant improvement in the growth of stems, maintaining the shoot–root ratio at the level observed in the control. This indicates that the applied doses of VC have an effect on the growth of larch seedlings that is entirely beneficial.

Table 1
Characteristics of control and vermicompost-amended soil (V10, V20) after 2 years of field *Larix decidua* seedlings vegetation.

Description and abbreviations of parameters		Control	V10	V20
Biometrics of seedling and mycorrhiza assessment[†]				
Stems height (S)	[cm]	$30.13^{c,\dagger}$	45.88^{b}	61.13^{a}
Stems diameter (D)	[mm]	4.50^{c}	8.50^{b}	12.00^{a}
Root length (R)	[cm]	13.63^{b}	22.88^{a}	26.63^{a}
R/S ratio	-	0.45	0.50	0.44
QI ratio (D2/(S/R))	-	9.16^{c}	36.03^{b}	62.73^{A}
Number of fine roots	[pcs]	4.10^{b}	5.90^{ab}	7.30^{a}
Length of fine roots	[mm]	29.50^{b}	61.40^{A}	88.60^{A}
Number of root tips	[pcs]	3.30^{b}	5.70^{a}	6.70^{a}
Autotrophic roots	[%]	70.73^{a}	16.95^{B}	4.11^{C}
Mycorrhizal roots	[%]	29.27^{b}	83.05^{A}	95.89^{A}
Root tips per fine roots	[pcs/1 mm]	8.90^{b}	10.80^{ab}	13.20^{a}
Mycorrhizal morphotypes of fungi	*Wilcoxina* [%]	66.67	79.55	68.49
	Tuber [%]	25.00^{a}	8.16^{b}	20.55^{a}
	Suillus [%]	8.33	10.20	10.96
Nematodes identification [indiv·100 cm^{-3}]				
Plant parasitic nematodes	*Merlinius*	5	3	3
	Bitylenchus	20	14	14
	Helicotylenchus	15	16	14
	Pratylenchus	10^{b}	5^{a}	4^{a}
	Paratylenchus	5	5	5
	Paratrichodorus	5	5	5
Bacteria feeders	*Rhabditis*	150^{a}	600^{B}	820^{B}
	Cylindrolaimus	65^{a}	180^{b}	190^{b}
	Plectus	55^{a}	120^{b}	150^{b}
Fungal feeders	*Aphelenchus*	80^{a}	160^{b}	240^{b}
	Aphelenchoides	60^{a}	90^{b}	120^{b}
	Ditylenchus	40	80	80
	Filenchus	30^{a}	90^{b}	110^{b}
Predatory nematodes	*Mylonchulus*	6^{a}	15^{b}	25^{b}
	Mononchus	3^{a}	5^{b}	10^{b}
	Prionchulus	3^{a}	7^{b}	8^{b}
	Clarkus	2^{a}	5^{b}	9^{b}
Total number of nematodes		554	1400	1807
Ecological indices	Simpson's domination	0.141	0.233	0.252
	Shannon's diversity	2.246	1.888	1.829
	Pielou's evenness	0.793	0.666	0.646

[†] Different letters indicate significant differences between variants at $p \leq 0.05$ (capital letters indicates significant differences between variants at $p \leq 0.01$ compared to control).

3.2. Mycorrhiza formation

The application of VC also increased the proportion of mycorrhizal roots and decreased the number of autotrophic roots. The role of mycorrhiza mycelium in the uptake of water and nutrients from the substrate and hyphal mantle in providing protection against phytopathogens in the rhizosphere is widely known and indisputable (Gupta et al., 2000; Varma, 2008). The size and conditions of fine roots in the VC variants in the test were better (Table 1). The mycorrhizal fine roots in the VC variants were 83% (V10) and 96% (V20) compared to 29% in the control. Recognized taxa of mycorrhizal morphotypes were different – with the endomycorrhizal *Wilcoxina*-like morphotypes prevalent in all variants (about 70%), especially in the V10 variant (nearly 80%), while the *Tuber*-like morphotypes were the most numerous in the control (25%) and significantly decreased in the V10 (8%), whereas *Suillus*-like morphotypes increased (by 2%) in both VC variants compared to the control. The most important observation was the apparent link between the improvement of the plant's condition after the addition of VC and the development of mycorrhizal fungi i.e. a much higher level of root mycorrhization and increasing *Suillus*-morphotypes. Klimek et al. (2011) and Leski et al. (2009) obtained similar results in improving mycorrhizal symbiosis in case of increasing organic matter by adding ectohumus and litter in forest nurseries.

3.3. Influence on nematode community

Other components of soil biota were also changed by VC. 17 taxa of nematode were identified to genus in the evaluated soils (Table 1). The largest quantity of nematode individuals was noted in the V20 variant – 1807 per·100 cm^{-3}, while their number in the control was only 554. In the V10 variant, 1400 individuals occurred per 100 cm^{-3}. As for plant parasitic nematodes, a significant reduction in abundance was observed only for *Pratylenchus* spp. The total number of plant parasitic nematodes in the VC variants decreased by about 20 individuals per 100 cm^{-3} of substrate. The abundance of bacteria feeder and fungal feeder nematodes increased significantly in the VC soils, except for *Ditylenchus* spp. The genera of *Rhabditis* spp., *Cylindrolaimus* spp., and *Aphelenchus* spp. in each of the substrates formed the class of eudominants. Besides, the proportion of *Rhabditis* spp. in the nematode community exceeded 40% in the VC soils. As a result of high populations of bacteria feeder and fungal feeder nematodes, one could expect to see a decrease in seedling disease complexes (Back et al., 2002). Predatory nematodes also increased in the VC soils. The values of the Shannon index decreased from 2.4 in the control to 1.9 in both VC soils. Dominguez et al. (2003)

and Gebremikael et al. (2016) have shown that earthworms have a strong influence on the nematode community, and our results for VC back this up.

The observed change in the nematode community of soils caused by VC can be assessed as favorable. Suppressing plant-parasitic nematodes and increasing free-living nematodes in plant rhizosphere leads to a significant improvement in the soil food chain and thus the health of soil and plants. Free-living nematodes are recognized as bioindicators of soil health because they can be used to determine the degradation pathways of dominant nutrients, as well as the structure of the soil food web and soil ecosystem functions (Ferris et al., 2012; Wang et al., 2014; You et al., 2018). As emphasized by You et al. (2018), a healthy soil food web should sustain nematodes with different life strategies and feeding behaviors ranging from fast-growing and fast-reproducing bacteria-feeding nematodes (colonizers) at the bottom of the soil food web to slow-reproducing but longer-living predaceous nematodes at the top of the soil food web. The application of VC should thus be integrated with other nematode management practices to improve sustainable crop protection.

3.4. Frequency and activity of bacteria and fungi

The abundance of most bacteria and fungi frequency depended significantly on the dose of VC added (Fig. S1). The most unexpected changes were observed in the proportion of bacteria of the Actinomycetes order, which accounted for 2.2% in the control and increased to 10.5% in V10, and to 5.4% in V20. Bacteria of the Pseudomonadaceae family showed a balanced prevalence in the control and V10 (about 2.3%); however their percentage dropped to 0.8% in V20. Correlations were found between the proportion of the *Bacillus* spp. and an increasing amount of VC ($r^2 = 0.93$) ranging from <0.01% in the control, to 2.2% in V10, and to 2.7% in V20. The Actinomycetes order formed the largest group, although the percentage of the entire order decreased and was lower than that of *Bacillus* spp. The latter's prevalence increased from 0% in the control to almost 3% in V20. Toxigenic *Penicillium* spp. and *Fusarium* spp. were suppressed by the increased population of *Bacillus* spp. (Ahemad and Kibret, 2014; Castaneda-Alvarez and Aballay, 2016; Hu et al., 2016). The results obtained were confirmed by the detection of functional genes (Fig. S1). The presence of genes of Pseudomonadaceae producing antibiotic *phlD* and *hcnAB* and Actinomycetes producing PPKS-I and NRPS was found in all the analyzed substrates. The only difference observed was in the case of five lipopeptides genes (i.e. *ituC*, *bacA*, *fenD*, *srfAA*, *bmyB*) characteristic of *Bacillus* spp. In the larch rhizosphere not treated with VC, these genes were not detected, while in the substrates with VC in both doses a positive result was obtained. The results clearly indicate the rhizosphere was inhabited by *Bacillus* spp., producing antimicrobial compounds, and thus affording enhanced protection against phytopathogens.

3.5. Effect of activity of enzymes

Addition of VC resulted in changes in soil enzyme activity (Table S3). In the case of the Effect index, there was an increase in the activity of acid phosphatases, inorganic pyrophosphatase, and urease in VC soils. A strong effect was also observed regarding the activity of dehydrogenases (1.767 and 2.131 for V10 and V20, respectively). Moreover, a correlation between a decrease in the Resistance index values along with an increase in the dose of VC was demonstrated. The Resistance index data showed that the addition of VC did not disturb rhizosphere homeostasis. Except for the activities of dehydrogenases and alkaline phosphatases, it can be concluded that the effect of VC was not significant. However, V10 showed better resistance than V20, similar to the results presented by Orwin and Wardle (2004). The enzyme activity values for all enzymes were correlated with an improvement in the values of microbiological, biometric and nematode parameters and increased mycorrhization. Many authors indicate the Resistance index to be one of the best indicators to assess the impact of various substances on the soil (Borowik et al., 2017; Telesiński et al., 2018). The addition of VC to the soil resulted in an increase in N content and improvement in the C:N ratio (Table S2), expressed in the form of an increase in microbial/enzymatic activity. In present experiment, in general, the V20 environment was slightly better in case of the Effect index, whereas V10 was better for the Resistance index, with this being seen most clearly based on the example of dehydrogenases activity.

3.6. Result of interactions of environmental components

The most important relationships between all the tested parameters are shown in Fig. 1. Correlations were found between some soil biota and the chemical properties of soils, grouped together into three sets. The values of the C:P, C:K ratios and $N\text{-}NH_4$ concentration were correlated with an abundance of *Bacillus* spp. ($r^2 = 0.89$-negative, $r^2 = 0.98$-positive and $r^2 = 0.99$-positive, respectively), while a correlation between the C:N ratio, as well as concentrations of $N\text{-}NH_4$, Mg, Zn and Fe, and the number of nematodes was also observed. The control variant showed a combination of high values of the following variables: autotrophic roots, plant parasitic nematodes, *Fusarium*, C:N, and to a lesser extent with *Penicillium* spp. and fungal feeders nematodes ($r = 0.99$). In turn, Set II, Set I, as well as dehydrogenases, mycorrhizal roots, C:P, and alkaline phosphatase were associated with the V10 and V20 variants. In addition, high values of *Clostridium* and *Wilcoxina*, as well as the Root length/Stems height ratio, were characteristic for the V10 variant.

It was noted that all the desirable biometric parameters of the seedlings increased along with the dose of VC and also with the both increasing number of total fungi, and mycorrhization. The variability of the C:N ratio was correlated with high *Fusarium* spp. load. However, the plant parasitic nematodes population was negatively correlated ($r^2 > 0.9$) with Set I and Set II, dehydrogenases, mycorrhization, C:P, alkaline phosphatase and $N\text{-}NH_4$. The ratios of carbon to N, P and K indicate that they are closely connected with enzymatic and biological activity. The observed reverse correlation between C:N and C:P, and P:K indicates that the shift of these ratio from unfavorable (above 100) to favorable (about 20) significantly modified not only the processes of decomposition and immobilization of organic matter.

The observed dependencies of bacteria-nematode and bacteria-nematoda-mycorrhization were closely correlated to the number of *Penicillium* spp. and fungal feeders nematodes. The most important observation was the apparent link between the improvement of the plant's condition after the addition of VC and the development of *Suillus* morphotype mycorrhizal fungi, bacteria feeders nematodes, and *Bacillus* spp. Other values, such as P, as well as total numbers of bacteria, *Tuber*, *Actinomycetes* and *Pseudomonas*, did not show a close relationship with a particular variant, although it can be seen that the proportion of the *Pseudomonas* and *Actinobacteria* species decreased in other bacterial groups, including *Bacillus* spp., along with a decrease in the C:N ratio, while the C:P and C:K ratios increased, and the efficiency index value of all enzymes tested increased in the experiment. At the same time, the setup described above helped to reduce harmful toxigenic *Penicillium*, *Fusarium*, autotrophic roots, plant parasitic nematodes, which points to the multithreaded and complex mechanism of establishing a balance in the rhizosphere of plants which is visible along with the delivery of highly valuable organic matter. After the addition of VC, *Bacillus* bacteria led to an increase in the number of types of lipopeptides, which as a result significantly reduced the abundance of phytopathogens and undesirable nematodes. Most of the key active substances are produced by rhizosphere-sporulating bacteria and species with a proven effect on plant parasitic nematodes including, for example, Pseudomonadaceae, *Bacillus* spp., *Brevibacillus* spp. and *Paenibacillus* spp. (Castaneda-Alvarez and Aballay, 2016; Topalović et al., 2020).

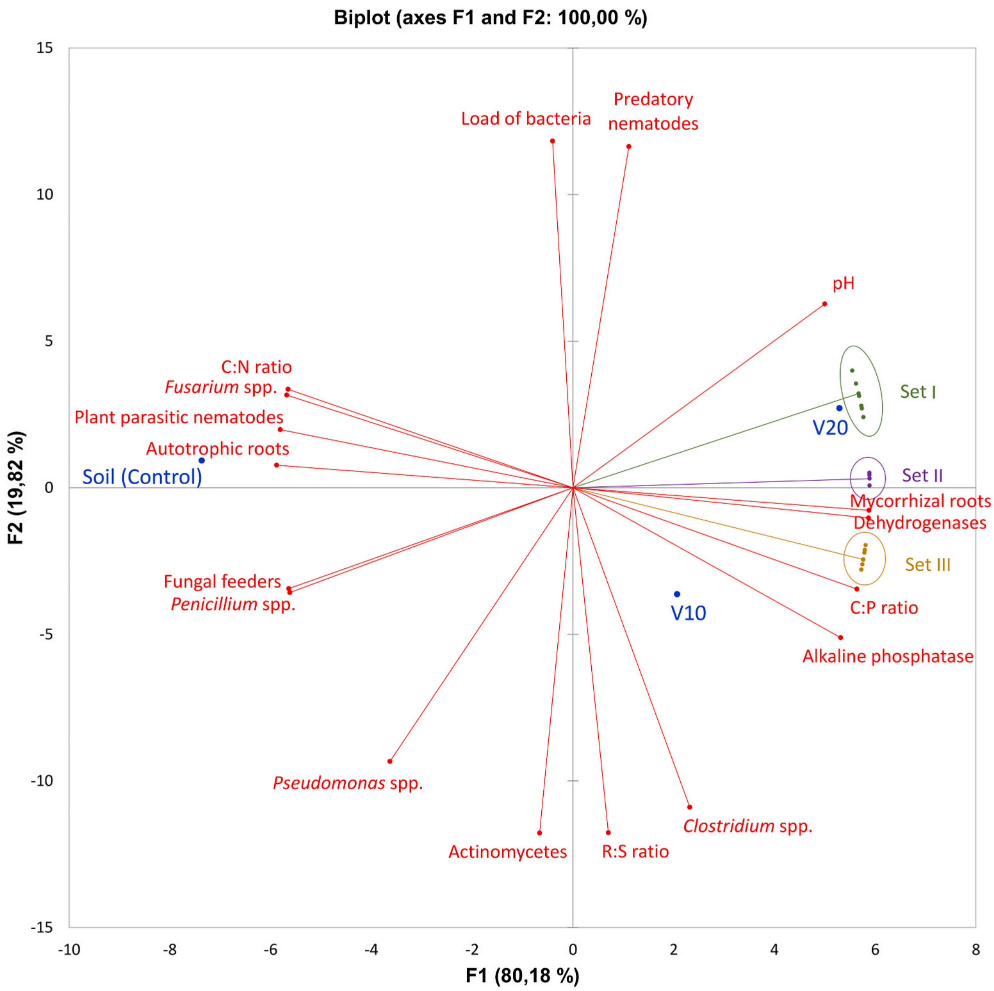

Fig. 1. Principal Component Analysis (PCA) of experimental factors for the control and the V10 and V20 samples. The lines represent the correlation coefficient between the principal component scores and each of the factors. Set I included: nitrate reductase, inorganic pyrophosphatase, acid phosphatases, C:K ratio, proteases, β-glucosidase, o-diphenol oxidase, urease, N-NH4 Set II included: *Bacillus* spp., *Suillus* mycorrhizal morphotype, Root length, Bacterial feeders, Number of root tips Set III included: Number of fine roots, Length of fine roots, Stems height, Stems diameter, Load of fungi, QI ratio, Root tips per fine roots, N-NO_3.

4. Conclusions

In this work, some correlations were found that have not been described before. Addition of *E. fetida* VC in two doses: 10% (V10) and 20% (V20) *v*/v to nursery soil resulted in beneficial changes in rhizospheric soil properties and consequently better growth of larch seedlings. In VC -incorporated soil, there was a clear relationship between the chemical changes and an improvement in the main soil microbiota/nematode parameters, the degree of mycorrhization and root biometrics as a result. The greatest positive impact was attributed to the increase in N-NH_4 content and the change in C:N ratio due to the introduction of VC. The addition of VC significantly increased the abundance of microbiota in the rhizosphere, especially *Bacillus* spp. producing lipopeptides, which in turn reduced the abundance of the community of negative bioindicators such as: toxigenic phytopathogens and plant parasitic nematodes. Considering the effect on improving plant growth, mycorrhization and root zone condition, the application of VC in tested dosages can be recommended as a sustainable practice in larch nursery management.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the anonymous reviewers for their valuable comments and suggestions. This work was financed by University of Warmia and Mazury in Olsztyn under Project Nos. 20.610.016-300 and 20.610.029-300.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2021.104101.

References

Abdelmagid, H.M., Tabatabai, M.A., 1987. Nitrate reductase activity of soils. Soil Biol. Biochem. 19, 421–427. https://doi.org/10.1016/0038-0717(87)90033-2.
Agerer, R., 2006. Fungal relationships and structural identity of their ectomycorrhizae. Mycol. Prog. 5, 7–107. https://doi.org/10.1007/s11557-006-0505-x.
Ahemad, M., Kibret, M., 2014. Mechanisms and applications of plant growth promoting Rhizobacteria: current perspective. J. King Saud Univ. Sci. 26, 1–20. https://doi.org/10.1016/j.jksus.2013.05.001.
Asghari, M., Rafiei, M., 2013. Germination and early growth of eucalyptus plants in commercial potting substrate amended with different rates of vermicompost. Glob. J. Med. Plant Res. 1 (1), 22–28.
Atik, A., Yılmaz, B., Aslan, F., 2015. Effects of three different concentrations of vermicompost on the growth in the with 2+0 aged seedlings of Anatolian black pine. Int. J. Agric. For. Fish. 3 (2), 25–31.
Back, M.A., Haydock, P.P.J., Jenkinson, P., 2002. Disease complexes involving plant parasitic nematodes and soilborne pathogens. Plant Pathol. 51, 683–697. https://doi.org/10.1046/j.1365-3059.2002.00785.x.
Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.-J., 2013. A review of

earthworm impact on soil function and ecosystem services. Eur. J. Soil Sci. 64, 161–182. https://doi.org/10.1111/ejss.12025.

Blouin, M., Barrere, J., Meyer, N., Lartigue, S., Barot, S., Mathieu, J., 2019. Vermicompost significantly affects plant growth. A meta-analysis. Agron. Sustain. Dev. 39, 34 doi:10.1007.

Borowik, A., Wyszkowska, J., Wyszkowski, M., 2017. Resistance of aerobic microorganisms and soil enzyme response to soil contamination with Ekodiesel ultra fuel. Environ. Sci. Pollut. Res. 24, 24346–24363. https://doi.org/10.1007/s11356-017-0076-1.

Cannell, M.G.R., Willett, S.C., 1976. Shoot growth phenology, dry matter distribution and root:shoot ratios of provenances of *Populus trichocarpa*, *Icea sitchensis* and *Pinus concorta* growing in Scotland. Silvae Genet. 25, 49–59.

Casida, L., Klein, D., Santoro, T., 1964. Soil dehydrogenase activity. Soil Sci. 98, 371–376.

Castaneda-Alvarez, C., Aballay, E., 2016. Rhizobacteria with nematicide aptitude: enzymes and compounds associated. World J. Microbiol. Biotechnol. 32, 203. https://doi.org/10.1007/s11274-016-2165-6.

Da Ronch, F., Caudullo, G., Tinner, W., de Rigo, D., 2016. In: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (Eds.), *Larix decidua* and Other Larches in Europe: Distribution, Habitat, Usage and Threats. European Atlas of Forest Tree Species. Publication Office of the European Union, Luxembourg, pp. 108–110. https://doi.org/10.2788/4251.

Danjon, F., Reubens, B., 2008. Assessing and analyzing 3D architecture of woody root systems, a review of methods and applications in tree and soil stability, resource acquisition and allocation. Plant Soil 303, 1–34. https://doi.org/10.1007/s11104-007-9470-7.

Dao, H.T.T., Seo, J.M., Hernandez, J.O., Han, S.H., Youn, W.B., An, J.Y., Park, B.B., 2020. Effective placement methods of vermicompost application in urban tree species: implications for sustainable urban afforestation. Sustainability 12 (14), 5822. https://doi.org/10.3390/su12145822.

Dick, W.A., Tabatabai, M.A., 1978. Inorganic pyrophosphatase activity of soils. Soil Biol. Biochem. 10, 59–65.

Dickson, A., Leaf, A.L., Hosner, J.F., 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. For. Chron. 36, 10–13.

Dominguez, J., Parmelee, R.W., Edwards, C.A., 2003. Interactions between *Eisenia andrei* (Oligochaeta) and nematode populations during vermicomposting. Pedobiologia (Jena) 47, 53–60. https://doi.org/10.1078/0031-4056-00169.

Edwards, C.A., 2004. Earthworm Ecology, second ed. CRC Press, Boca Raton. https://doi.org/10.1201/9781420039719.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606. https://doi.org/10.1016/0038-0717(88)90141-1.

Ferris, H., Griffiths, B.S., Porazinska, D.L., Powers, T.O., Wang, K.-H., Tenuta, M., 2012. Reflections on plant and soil nematode ecology: past, present and future. J. Nematol. 44, 115–126.

Gebremikael, M., Steel, H., Buchan, D., Bert, W., De Neve, S., 2016. Nematodes enhance plant growth and nutrient uptake under C and N-rich conditions. Sci. Rep. 6, 32862. https://doi.org/10.1038/srep32862.

Gupta, V., Satyanarayana, T., Garg, S., 2000. General aspects of mycorrhiza. In: Mukherji, K.G., Chamola, B.P., Singh, J. (Eds.), Mycorrhizal Biology. Kluwer, Dordrecht, pp. 27–44.

Hirano, T., Suzuki, K., Hirata, R., 2017. Energy balance and evapotranspiration changes in a larch forest caused by severe disturbance during an early secondary succession. Agric. For. Meteorol. 232, 457–468. https://doi.org/10.1016/j.agrformet.2016.10.003.

Hu, J., Wei, Z., Friman, V.-P., Gu, S.-H., Wang, X.-F., Eisenhauer, N., Yang, T.-J., Ma, J., Shen, Q.-R., Xu, Y.-C., Jousset, A., 2016. Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression. mBio 7 (6). https://doi.org/10.1128/mBio.01790-16 e01790-16.

Kaczyńska, G., Borowik, A., Wyszkowska, J., 2015. Soil dehydrogenases as an Indicator of contamination of the environment with petroleum products. Water Air Soil Pollut. 226, 372. https://doi.org/10.1007/s11270-015-2642-9.

Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol. Fertil. Soils 6, 68–72. https://doi.org/10.1007/BF00257924.

Klimek, A., Rolbiecki, S., Rolbiecki, R., Hilszczańska, D., 2011. The effect of organic fertilization and mulching on the growth of European larch (*Larix decidua* L.) and soil biological activity. For. Res. Pap. 72, 233–239. https://doi.org/10.2478/v10111-011-0023-8.

Klimek, A., Rolbiecki, S., Rolbiecki, R., Kowalska, A., 2013. Comparison of the effects of mulching with ectohumus and controlled micorrhization on plants and mites (Acari) in the container production of Scots pine seedlings, in Polish. Infrastruct. Ecol. Rural Areas 3 (1), 37–50.

Kowalczyk, J., Neyko, I., 2011. The breeding value of selected families of European larch (*Larix decidua* Mill.) of Sudeten origin growing on an experimental plot in Zwierzyniec Lubelski (in Polish). For. Res. Pap. 72 (3), 213–224. https://doi.org/10.2478/v10111-011-0021-x.

Kumar, Raj, Shamet, G.S., Alam, N.M., Jana, Chayna, 2016. Influence of growing medium and seed size on germination and seedling growth of *Pinus gerardiana* wall. Compost Sci. Util. 24 (2), 98–104. https://doi.org/10.1080/1065657X.2015.1048906.

Ladd, J.N., Butler, J.H.A., 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem. 4, 19–30. https://doi.org/10.1016/0038-0717(72)90038-7.

Lazcano, C., Sampedro, L., Zas, R., Domínguez, J., 2010a. Assessment of plant growth promotion by vermicompost in different progenies of maritime pine (*Pinus pinaster* Ait.). Compost Sci. Util. 18, 111–118. https://doi.org/10.1080/1065657X.2010.10736943.

Lazcano, C., Sampedro, L., Zas, R., Domínguez, J., 2010b. Vermicompost enhances germination of the maritime pine (*Pinus pinaster* Ait.). New For. 39, 387–400. https://doi.org/10.1007/s11056-009-9178-z.

Leski, T., Rudawska, M., Aučina, A., Skridaila, A., Riepšas, E., Pietras, M., 2009. Influence of pine and oak litter on growth and mycorrhizal community structure of scots pine seedlings in bare-root nursery conditions. Sylwan 153, 675–683.

Medina-Sauza, R.M., Álvarez-Jiménez, M., Delhal, A., Reverchon, F., Blouin, M., Guerrero-Analco, J.A., Cerdán, C.R., Guevara, R., Villain, L., Barois, I., 2019. Earthworms building up soil microbiota, a review. Front. Environ. Sci. 7, 81. https://www.frontiersin.org/article/10.3389/fenvs.2019.00081.

Meng, S.W., Jia, Q.Q., Zhou, G., Zhou, H., Liu, Q.J., Yu, J., 2018. Fine root biomass and its relationship with aboveground traits of *Larix gmelinii* trees in northeastern China. Forests 9, 35. https://doi.org/10.3390/f9010035.

Migliavacca, M., Cremonese, E., Colombo, R., Busetto, L., Galvagno, M., Ganis, L., Meroni, M., et al., 2008. European larch phenology in the Alps: can we grasp the role of ecological factors by combining field observations and inverse modelling? Int. J. Biometeorol. 52, 587–605. https://doi.org/10.1007/s00484-008-0152-9.

Niu, X., Zhai, P., Zhang, W., Gu, Y., 2019. Effects of earthworms and agricultural plant species on the soil nematode community in a microcosm experiment. Sci. Rep. 9, 11660. https://doi.org/10.1038/s41598-019-48230-0.

Orwin, K.H., Wardle, D.A., 2004. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biol. Biochem. 36, 1907–1912. https://doi.org/10.1016/j.soilbio.2004.04.036.

Pascual, J.A., Ceglie, F., Tuzel, Y., Koller, M., Koren, A., Hitchings, R., Tittarelli, F., 2018. Organic substrate for transplant production in organic nurseries. A review. Agron. Sustain. 38, 35. https://doi.org/10.1007/s13593-018-0508-4.

Pathma, J., Sakthivel, N., 2012. Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. SpringerPlus 1, 26. https://doi.org/10.1186/2193-1801-1-26.

Perucci, P., Casucci, C., Dumontet, S., 2000. An improved method to evaluate o-diphenol oxidase activity of soil. Soil Biol. Biochem. 32, 1927–1933. https://doi.org/10.1016/S0038-0717(00)00168-1.

Seinhorst, J.W., 1962. On the killing fixation and transferring to glycerin of nematodes. Nematologica 8, 29–35. https://doi.org/10.1163/187529262X00981.

Shuman, J.K., Shugart, H.H., O'Halloran, T.L., 2011. Sensitivity of Siberian larch forests to climate change. Glob. Chang. Biol. 17 https://doi.org/10.1111/j.1365-2486.2011.02417.x.

Skwiercz, A.T., Adamiak, E., Stefanovska, T., Szelągowska, P., Zatoń, K., Sobolewska, M., 2018. Nematodes in the soil and roots of spring barley grown in crop rotation and long-term monoculture. Acta Sci. Pol. Agric. 17, 115–124. https://doi.org/10.37660/aspagr.2018.17.3.1.

Song, X., Liu, M., Wu, D., Griffiths, B.S., Jiao, J., Li, H., Hu, F., 2015. Interaction matters: synergy between vermicompost and PGPR agents improves soil quality, crop quality and crop yield in the field. Appl. Soil Ecol. 89, 25–34. https://doi.org/10.1016/j.apsoil.2015.01.005.

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1.

Telesiński, A., Krzyśko-Łupicka, T., Cybulska, K., Wróbel, J., 2018. Response of soil phosphatase activities to contamination with two types of tar oil. Environ. Sci. Pollut. Res. 25, 28642–28653. https://doi.org/10.1007/s11356-018-2912-3.

Thakuria, D., Schmidt, O., Finan, D., Egan, D., Doohan, F.M., 2010. Gut wall bacteria of earthworms: a natural selection process. ISME J. 4 (3), 357–366. https://doi.org/10.1038/ismej.2009.124.

Topalović, O., Hussain, M., Heuer, H., 2020. Plants and associated soil microbiota cooperatively suppress plant-parasitic nematodes. Front. Microbiol. 11, 313. https://doi.org/10.3389/fmicb.2020.00313.

UNFCCC, 2015. Adoption of the Paris agreement FCCC/CP/2015/L.9/rev.1. https://unfccc.int/resource/docs/2015/cop21/eng/l09r01.pdf.

Van Groenigen, J.W., Lubbers, I.M., Vos, H.M., Brown, G.G., De Deyn, B.G., van Groenigen, J.K., 2014. Earthworms increase plant production: a meta-analysis. Sci. Rep. 4, 6365. https://doi.org/10.1038/srep06365.

Varma, A. (Ed.), 2008. Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-physiology, Structure and Systematics. Springer-Verlag, Berlin, Heidelberg.

Wang, K.-H., Radovich, T., Pant, A., Cheng, Z., 2014. Integration of cover crops and vermicompost tea for soil and plant health management in a short-term vegetable cropping system. Appl. Soil Ecol. 82, 26–37. https://doi.org/10.1016/j.apsoil.2014.05.003.

You, X., Tojo, M., Ching, S., Wang, K.H., 2018. Effects of vermicompost water extract prepared from bamboo and kudzu against *Meloidogyne incognita* and *Rotylenchulus reniformis*. J. Nematol. 50 (4), 569–578. https://doi.org/10.21307/jofnem-2018-054.