Chapter 18
Impact of Green Manure and Vermicompost on Soil Suppressiveness, Soil Microbial Populations, and Plant Growth in Conditions of Organic Agriculture of Northern Temperate Climate

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18.1 Introduction

Several aspects of agricultural management regime, such as crop rotation, tillage frequency, compost or manure type, application of pesticides and synthetic fertilizers, and water regime, are key determinants of microbial community structure in the soil. Vegetation is also an important factor since plants are providing soil

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M.K. Meghvansi, A. Varma (eds.), *Organic Amendments and Soil Suppressiveness in Plant Disease Management*, Soil Biology 46,
DOI 10.1007/978-3-319-23075-7_18

microorganisms with specific carbon sources (Garbeva et al. 2004), but, on the other hand, microbial products can influence the decomposition of organic matter in the soil (Lützow et al. 2006). Several investigations show long-term positive influence of organic farming practices on soil quality and microbiological activity in comparison with conventional farming, due to regular crop rotation, and the absence of synthetic fertilizers and pesticides (Shannon et al. 2002). Fertilizing the soil rather than the plant is an organic farmer's goal to assure sufficient nutrient mineralization (Fliessbach and Mader 2000). In the meta-analysis of several investigations about the impact of organic agriculture on soil organisms, it was concluded that soil fungal populations mostly respond positively to organic management, but effects on microbial biomass and activity have been contradictory (Bengtsson et al. 2005).

The objective of this study was to provide an analysis of the impact of organic amendments, i.e., green manure and vermicompost, on the soil microorganisms and plant growth and health in conditions of organic agriculture of Northern temperate climate.

Some case studies dealing with green manure or vermicompost amendments in organic agriculture are discussed giving deeper analyses of the vermicompost impact on plant growth. The first case study is about the impact of green manure on soil microbial populations and soil suppressiveness against such pathogens as late blight, potato scab, and black scurf of potato in organic agriculture. The second case study is about the use of vermicompost in organic starch potato cultivation. Vermicompost produced from composted grass and starchless potato pulp was amended in the field experiment in two growing seasons. The development and severity of the late blight were assessed, as well as the impact on several groups of soil microorganisms. During the growing season, the plant response to the vermicompost amendments was monitored in the terms of photosynthetic activity and leaf chlorophyll content. The possible acting mechanisms of the vermicompost on plant growth are also discussed.

18.2 Green Manure

18.2.1 Impact of Green Manure on the Soil Biochemical and Microbiological Properties and Plant Parameters

Truu et al. (2008) studied a set of microbiological and biochemical properties of soil to assess the influence of agricultural practices on the three most widespread soil types (calcaric regosols, calcaric cambisols, and stagnic luvisols) in the fields of horticultural farms throughout Estonia. Investigation showed that soils managed according to organic farming principles were generally characterized by elevated microbiological parameter (microbial activity and biomass) values, but at the same time the variation of those parameters among soils from these fields was also highest. Researchers offer an opinion that the reason for such large deviations may be the different durations of organic management practice as well as differences in management history among fields, such as different amount and types of organic fertilizers (green or brown manure) applied and differences in crop rotation. Truu et al. (2008) also found that legume-based (mainly clover) crop rotation increased soil respiration and microbial biomass.

In an investigation in the semiarid Canadian prairie comparing annual legumes as green manure (green fallow) with tilled fallow–wheat and continuing wheat cultivation, it was estimated that after 6 years of these management practices, significant improvements were detected in several microbiological characteristics such as colony counts of aerobic bacteria and filamentous fungi. Four green manure crops, black lentil (*Lens culinaris* Medikus), tangier flat pea (*Lathyrus tingitanus* L.), chickling vetch (*Lathyrus sativus* L.), and feed pea (*Pisum sativum* L.), were used. This investigation also proved that the microbiological attributes of the soil are sensitive and responsive to the beneficial influence of the particular cropping systems (Biederbeck et al. 2005).

It is reported that organic farming with various cover crops and green manure in combination with animal manure in the long term results in higher biodiversity of soil organisms. The diversity of bacterial functional communities has been recorded to be higher in soils from organic farms, while species diversity was similar (Liu et al. 2007). Higher abundance and diversity of actinomycetes, important decomposers of organic material, is reported in organic tomato fields (manured with leguminous green manures and/or organic soil amendments) than conventional ones in Mediterranean climate (Drinkwater et al. 1995). The ratios of Gram-positive to Gram-negative bacteria and of bacteria to fungi have been reported to be higher in the fields with organic treatments (plant residues and straw incorporated into the soil) than in the conventional treatments (Marschner et al. 2003).

In an investigation in Maine (USA), it has been observed that green manure (rapeseed) has increased the total population of cultivable bacteria, mainly Gram-negative bacteria in organic farming system and Gram-positive bacteria in conventional farming system (Bernard et al. 2012).

Edesi et al. (2013) studied the influence of organic cultivation with green manure and cattle manure, organic cultivation with green manure, and conventional cultivation with green manure, cattle manure, mineral fertilizers and pesticides on soil microbial activity, and plate count microorganisms in podzoluvisol in Estonia. They found that the total number of bacteria was not different under various management regimes. All soil samples were examined for molds, yeasts, mesophilic spore-forming bacteria, *Fusarium* spp., actinomycetes, azotobacteria, cellulose decomposers, and denitrifying and nitrifying bacteria. In this investigation, the abundance of abovementioned groups of microorganisms did not differ significantly among treatments with exception of nitrifying bacteria. The amount of nitrifying bacteria was higher in both organic and conventional systems treated with cattle manure than in organic cultivation system treated only with green manure. Researchers conclude that although the green manuring is considered to be an important management practice in organic farming to maintain and increase soil microbial activity and the abundance of microbes in different microbial populations, it is important to use also other organic fertilizers such as animal manure in addition to green manure (Edesi et al. 2013).

Cover crops have traditionally been used to reduce soil erosion and build soil quality, but more recently cover crops are being used as an effective tool in organic weed management. Wortman et al. (2013) demonstrated that weeds may alter soil microbial community structure as a means of increasing competitive success in arable soils. However, the relationship between weeds and soil microbial communities requires further investigations.

Tein et al. (2014) investigated how different farming systems influence tuber yields and quality of potato as well as how potato cultivation within a crop rotation under different farming systems affects soil quality. Experiments were carried out on stagnic luvisol in Estonia. In this study, potato was part of a five crop rotation experiment in which red clover (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), peas (*Pisum sativum* L.), potato, and barley (*Hordeum vulgare* L.) followed each other simultaneously on a same field. In the first organic farming system, catch crops were used to provide organic green manure. In the second organic system, a fully composted cattle manure at a rate of 40 tons/ha was also added as a fertilizer. It was estimated that the first system significantly decreased the average soil potassium (K) concentration after potato cultivation. The second system significantly increased the average soil organic carbon (C) and phosphorus (P) concentrations after potato cultivation. The fresh tuber yield differences between both systems were found to be nonsignificant. There were no significant differences among both systems in average tuber K, calcium (Ca), dry matter, and starch concentrations.

Green manure can be incorporated in the soil as a fresh plant material or processed. Direct incorporation of red clover-derived slurry and compost (both with equal nitrogen (N) and C in comparison to fresh red clover) in the leek field in Sweden resulted in the immediate increase in the abundance of bacteria and fungi (estimated according to fatty acid analysis). Mulching with fresh red clover sustained a higher bacterial and fungal biomass until the end of the cropping season and stimulated arbuscular mycorrhizal fungi (estimated as amount of neutral lipid fatty acid 16:1ω5) at the end of the cropping season (Elfstrand et al. 2007). Although in another investigation in Sweden various N amendments were used for 53 years, it was found out that soil fungal populations did not differ among treatments, including the treatment with green manure (fodder crops) every second year (Börjesson et al. 2012). The protease, acid phosphatase, and arylsulphatase activities were highest in the direct incorporation treatment, whereas enzyme activity in treatments with processed red clover was never higher than in the control treatments. There were no differences in leek harvest yield, but the N, P, and sulfur (S) concentrations in the leek crop at harvest increased in response to higher amounts of slurry and compost amendment. The authors concluded that direct incorporation of a red clover ley before planting of the leek was most effective for enhancing and sustaining a high microbial biomass and high rates of enzyme activity in the soil in comparison to other treatments: mulching with fresh red clover, incorporation of biogas slurry from fermented red clover and composted red clover (Elfstrand et al. 2007).

Arlauskiene et al. (2013) presented the analysis of application of grass biomass in organic manure production using innovative technologies, i.e., after additional mulching. Field experiments with different methods of perennial grasses (festulolium, red clover, and lucerne) aboveground biomass removed from the field mulching four times during the period of vegetation and mixed—first cut removed from the field, second, and third—mulching for green manure) were carried out in Lithuania on an endocalcari–endohypogleyic cambisol. As a result, the mulch of grasses was partially mineralized. Late in the autumn, $N_{inorganic}$ content in soil increased the least after application of the aboveground mass of grasses in a combined manner. It was concluded that it is purposeful to apply the aboveground mass of perennials in a combined manner from the environmental approach because the mulch of perennials affects the soil $N_{inorganic}$ content in spring more than in the autumn.

Olesen et al. (2009) studied the influence of green manure on the yield of winter cereal in organic arable farming on three different soil types varying from coarse sand to sandy loam in Denmark. All cuttings of the grass–clover were left on the soil as the mulch. Catch crops did not significantly affect grain yield and total aboveground biomass but reduced grain N concentration for 0.4–0.5 N kg^{-1} dry matter. The authors are of the opinion that the slower mineralization of the organic matter in the incorporated grass–clover probably increased late season N uptake, thereby primarily affecting grain protein content. The dry matter biomass in catch crops was considerably smaller than the weed biomass. The dominating leaf diseases for winter wheat were Septoria, mildew, and stripe rust. The dominating leaf diseases on winter rye were rye leaf rust and scald. There was no significant relationship between disease severity and grain yield, when yield was corrected for effects of year and N input. The results obtained by Olesen et al. (2009) showed that N in grass–clover green manure crops can be an important source of N for winter cereals on soils with good N retention, but they should be avoided on sandy soils with high rates of N leaching.

Results provided by Doltra and Olesen (2013) indicate that in Nordic climates, legume-based catch crops can contribute to the ecological intensification of spring cereals, not only by reducing the nitrate leaching and increasing N retention but also by improving yields.

However, investigations about soil fungal communities do not clearly indicate that they are always positively influenced by organic agriculture practices. In an investigation in southern Germany, it was determined by the cultivation-independent approach that fungal populations were almost entirely uninfluenced by the farming management practices, whereas active population, investigated by the isolation of hyphae using a soil-washing technique, showed a clear response to farming management practices (Hagn et al. 2003). The propagule number of *Trichoderma* has been shown to be higher in soils from conventional farms that used animal manure with synthetic fertilizers in comparison with organic farms using animal manure and deep litter (Elmholt and Labouriau 2005), but it depended on the year of analyses. It is assumed that *Trichoderma* spp. are less affected by a soil disturbance (after the use of pesticides) than other soil fungi and are able to quickly colonize niches left by other organisms in conventional fields with monoculture (Liu et al. 2007). In an investigation in Denmark, it was determined that there were no significant differences of amount of cultivable filamentous fungi and yeasts among organically cultivated fields and fields with synthetic fertilizer and/or animal manure. There were differences only in the abundance of particular genera, i.e., *Penicillium* spp. and *Gliocladium roseum* were more represented under organic than conventional farming (Elmholt and Labouriau 2005).

In microcosm studies with various types of manure, including green manure (grass–clover), it was detected that fresh grass–clover amendment to the soil increased several times the easy degradable organic carbon content, microbial biomass, and significant changes in microbial diversity measures compared to the raw cattle slurry and the two anaerobically digested materials (cattle slurry/maize, cattle slurry/grass–clover). At the same time, the increased microbial biomass depleted the soil for mineral nitrogen (Johansen et al. 2013). Soil microbial parameters alone do not give broad understanding about the soil quality. For agricultural purposes, it is important to reduce the level of soilborne fungal and bacterial pathogens.

Two classical types of soil suppressiveness to soilborne plant pathogens are known (Weller et al. 2002). General suppression owes its activity to the total microbial biomass and is not transferable between soils. Specific suppression owes its activity to the effects of select groups of microorganisms and is transferable. Take-all decline results from the building of fluorescent *Pseudomonas* spp. that produce the antifungal metabolite 2,4-diacetylphloroglucinol. Producers of this metabolite may have a broader role in disease-suppressive soils worldwide (Weller et al. 2002).

Disease-suppressive properties of the soil depend on various factors: soil texture, structure, pH, Ca content, agricultural practices (crop rotation, tillage, fertilizers, and organic amendments), and soil biota (microbial activity or soil respiration, microbial community diversity and composition, population size of particular microbial groups like actinomycetes) (Postma et al. 2008). The soil can act as a reservoir of the inoculum of pathogenic fungi, for example, oospores of late blight *Phytophthora infestans* can survive in the soil in the absence of the host for several years (Drenth et al. 1995). In order to estimate the impact of agricultural practices, it is important to evaluate both soil microbial parameters and disease-suppressive capacity of the soil.

Brassica crops used in crop rotations and as green manures have been associated with reductions in soilborne pests and pathogens. These reductions have been attributed to the production of volatile sulfur compounds through a process known as biofumigation and to changes in soil microbial community structure (Larkin and Griffin 2007). It is reported that green manure from white mustard (*Sinapis alba*), oriental mustard (*Brassica juncea*), and a sorghum–sudangrass hybrid in Newport (USA) reduced the verticillium wilt in the subsequent potato crop. The mustard mixture reduced also other diseases—black scurf and common scab (Larkin et al. 2011a) and, in other investigation, also the rhizoctonia stem canker of potato (Larkin et al. 2011b). Green manure from rye and vetch reduced the incidence of southern blight of tomatoes caused by *Sclerotium rolfsii* (Bulluck III and Ristaino 2002). Many vegetables, primarily the family *Brassicaceae*, are rich in glucosinolates (beta-thioglucoside-*N*-hydroxysulfonates), the precursors of isothiocyanates, and/or their breakdown products known for their fungicidal, nematocidal, and allelopathic properties (Fahey et al. 2001). Lord et al. (2011) assessed the effects of brassica green manures on pale potato cyst nematode *Globodera pallida*. Three *Brassica juncea* lines containing high concentrations of 2-propenyl glucosinolate were the most effective, causing over 95 % mortality of encysted eggs of *G. pallida* in the polyethylene-covered soil. The toxic effects of green manures were greater in the polyethylene-covered than in open soil. In this research, toxicity in the soil correlated with the concentration of isothiocyanate-producing glucosinolate but not total glucosinolate in green manures. However, disease reductions are not always associated with higher glucosinolate-producing crops and have been also observed with non-*Brassica* crops (barley and ryegrass), indicating other mechanisms and interactions are important, particularly for control of *Rhizoctonia solani* (Larkin and Griffin 2007).

18.2.2 Case Study

Only a small part of soil fungi (17 %) and bacteria (0.1–1 %) (Bridge and Spooner 2001; Torsvik et al. 1996; Val-Moraes et al. 2013) are cultivable, and therefore, currently two approaches are used to analyze soil microbial communities, i.e., conventional plating of cultivable microorganisms and DNA-based analyses that are independent of cultivation. Amplified rRNA gene restriction analysis (ARDRA) gives genetic fingerprinting of communities, populations, or phylogenetic groups. In soil microbiology, this method is used to determine the diversity within phylogenetic or functional groups of microorganisms (Lynch et al. 2004). Several studies have shown that quantitative PCR can be used successfully to determine the abundance of specific groups of microorganisms in the soil. An important genus of soil fungi analyzed with this method is *Trichoderma* that is known for its antagonistic activities against plant pathogens (Cordier et al. 2006).

The objective of our study (Grantina et al. 2011) was to conduct complex investigation of microbial attributes in the soil of three organic and four conventional agriculture fields in order to estimate the impact of 6-year-long organic agriculture practices in Northern temperate zone conditions and to compare the characteristics of microbial populations with crop plant health and pathogen suppression. For the characterization of soil bacteria, only classical microbiological methods that analyze cultivable bacteria were used, but soil fungal populations were assessed using both classical and molecular biology methods targeting also those organisms that are uncultivable under laboratory conditions. The hypothesis was that 6 years of organic agriculture practices after long-term conventional agriculture can result in some improvements in the conditions of soil microbial populations and/or plant health and pathogen suppression. Three fields of organic agriculture and four fields of conventional agriculture were examined at the State Priekuli Plant Breeding Institute. Fields of organic agriculture were treated with this type of management for 6 years. The crop rotation in organic fields was as follows: spring crops with clover undersown, clover, winter crops, potatoes, and crucifers (*Brassicaceae*) for green manure and spring crops. The green manure was incorporated in each field every 6 years. In other years, the amelioration of the soil was achieved by cultivating the clover (symbiotic nitrogen fixation), as well as with turning the plant residues into the soil. Similar to organic fields, in the conventional fields, winter crops were grown before potatoes. In all analyzed fields, there was sod-podzolic soil. Soil pH and soil moisture contents were similar in organic and conventional agriculture fields.

Soil samples were taken in the fields in June and in August 2008 and 2009. Nine subsamples were collected on transect of each field at a sampling depth of 10–15 cm (three subsamples in each corner of the field and three subsamples in the middle of the field, 100 g each). The subsamples were pooled together to create three larger samples for every field. Altogether, 84 soil samples were analyzed. The information about the time of outbreak and severity of late blight (*Phytophthora infestans*), potato scab (*Streptomyces scabies*), and black scurf of potato (*Rhizoctonia solani*) was recorded each growing season. On average, the total number of bacteria was significantly higher in organic agriculture fields in comparison with conventional fields. The increase of bacterial colony-forming units (CFU) was on average approximately 70 %. There was a trend that at the end of summer 2008, the number of Actinobacteria in all fields decreased (except one organic field with green manure and cover crops in this year), but in 2009 the number of Actinobacteria increased in all fields; however, these changes were not statistically significant. Overall, the total number of Actinobacteria was significantly higher in organic agriculture fields—on average almost four times if results of both years are combined. The total number of yeasts and maltose-utilizing bacteria was fluctuating during the analyzed period, and on average it was higher in samples of 2009 and also in organic agriculture fields in general in comparison to conventional fields—on average by 190 % (statistically not significant).

The ratio of bacteria to fungi differed significantly in particular sampling times. On average, the ratio of bacteria to fungi was significantly higher in the conventional fields (498 vs. 312). A common trend was observed that the total number of cultivable filamentous fungi (CFF) increased in 2009 in all fields with the exception of conventional barley field. It is still unclear, why the total number of CFF increased significantly in the second year in almost all fields, since none of the factors included in the regression models explained this shift. In spite of the fact that one conventional field received fungicides (mancozeb and others) several times during the second summer, the total number of CFF was increased 9.5 times at the end of August 2009 in comparison with the previous level. Data about dominating CFF genera showed that especially the number of CFU of *Mucor* spp. and sterile mycelia increased in 2009, while members of other genera remained unchanged. It contradicts other investigations that found that the application of such fungicide as mancozeb in amount of 10 mg kg^{-1} in soil decreased the amount of fungi for at least 3 months (Doneche et al. 1983), although the concentration of mancozeb applied on the abovementioned conventional field was significantly lower. In general, the total number of CFF was significantly higher in organic fields. The increase of CFF numbers in organic agriculture fields was on average approximately by 110 %.

Changes in the abundance of dominant fungal genera (*Trichoderma*, *Mucor*, *Mortierella*, *Penicillium*, and *Verticillium*) and sterile mycelia (not sporulating after 10 days of incubation) were evaluated in the two-year period. Similar to the investigations of Liu et al. (2007), in our investigation there were no statistically significant differences in the propagule numbers of *Trichoderma* genus among fields of organic and conventional agriculture. The most abundant genus was *Penicillium*—on average 37.8 ± 14.4 % of all fungi, while other genera were represented by 5–10 % of all CFF, and sterile mycelia covered 33.0 ± 10.1 %. In organic fields, only propagule numbers of *Penicillium* and *Verticillium* were significantly higher than in conventional fields. Higher numbers of *Penicillium* have been recorded in organic fields amended with animal manure and deep litter in the work of Elmholt and Labouriau (2005). Other genera were similarly abundant in both groups of fields.

Consequently, in our investigation we found that colony counts of all groups of cultivable microorganisms (total bacterial count, Actinobacteria, yeasts and maltose-utilizing bacteria, and CFF) were significantly higher in organic agriculture fields after a 6-year-long period of organic agriculture practices than in continued conventional fields. This is in line with the results of Biederbeck et al. (2005) in the semiarid Canadian prairie after the period of 6 years. Similarly, two times higher bacterial numbers under low-input (integrated) agriculture in comparison to high-input agriculture have been recorded in an investigation in the Netherlands (Bloem et al. 1992). There were no statistically significant differences among fields of organic and conventional agriculture for the results obtained by molecular methods, although the mean Shannon diversity index of fungal population was higher in the organic fields in comparison to the conventional agriculture fields (2.56 vs. 2.43). Similar to our study, no significant differences were detected between the two agricultural regimes (organic farms with ecological or biodynamical practices and conventional farms) regarding the number of phylotypes per field and Shannon diversity indices of arbuscular mycorrhizal fungi in onion fields in the Netherlands using molecular methods (Galván et al. 2009).

Quantitative PCR indicated an increase in the amount of *Trichoderma* spp. DNA in 2009, especially in August. However, there were no statistically significant differences among fields of organic and conventional agriculture, although the mean values of this parameter were higher in organic fields, i.e., 9.23 ng g^{-1} dry soil vs. 7.17 ng g^{-1} dry soil. In 2008, the first damage of the late blight (*Phytophthora infestans*) in organic fields was observed 7–10 days earlier than in conventional fields. Late blight significantly destroyed foliage (30–100 %) in organic field 10–14 days before it reached such level in conventional fields. In

2009, the first spots of the disease on potato leaves were observed at the same time on both environments, but significant foliage damages (5–100 %) were assessed after 10 days in organic field and only after 24 days in conventional field. The application of fungicide delayed the late blight development in conventional field and saved crop vegetation for longer time. The late blight development was faster in 2008 than in 2009 due to more favorable weather conditions (more rainfall during August) in 2008. The precipitation in August 2009 was approximately two times less than in two previous years. The prevalence of potato scab caused by *Streptomyces scabies* and black scurf of potato caused by *Rhizoctonia solani* was similar in the fields of both agricultural practices. Consequently, in contrast to the soil microbiological indicators that showed improvement after 6 years of organic agricultural practices in comparison to the conventional agricultural fields, the plant health, in terms of plant disease suppression, had not been improved. Controversial results about the capacity of low tillage and organic agriculture systems to reduce the disease levels, for example, of common root rot of cereals caused by *Cochliobolus sativus*, verticillium wilt, and common scab of potato, have been obtained in previous investigations (Bailey and Lazarovits 2003). Fungal activity measured as fungal biomass has been proved to correlate with *R. solani* suppression in soil (Postma et al. 2008). Our investigation showed that the increase in the number of CFF did not result in the disease suppression, possibly because a 6-year organic management period was too short to reduce the plant pathogen levels in the soil, and crop rotation had gone through the whole cycle only once.

18.3 Vermicompost

18.3.1 Impact of Vermicompost on Plant Growth

The use of vermicompost in agriculture is increasing. Among beneficial effects of vermicompost in agriculture, it is usually generally stated that vermicompost application leads to the improvement of soil's physical properties, including porosity, water retention capacity, etc. (Ferreras et al. 2006). However, in short-term studies in controlled conditions, soil mechanical properties are of less importance in comparison to field experiments. Therefore, potential beneficial effect from vermicompost application could be more easily related to changes in the chemical composition of substrate, e.g., mineral nutrients and plant hormonelike substances. Within the present review, instead of analyzing agronomic properties, we will focus on direct and indirect physiological effects of vermicompost on plants.

An overview of possible direct or indirect physiological effects of vermicompost on plants is given in Table 18.1. Due to a different degree of mineralization and variation in mineral nutrient content in feeding material, it is evident that the beneficial effect of vermicompost needs to be analyzed at least at two levels of soil mineral nutrient availability. In conditions of low mineral supply, plant growth

Table 18.1 Possible direct and indirect physiological effects of vermicompost constituents on plants

Constituent	Concentration or level	Possible benefits	Possible negative consequences
Minerals	Relatively low, variable, and unbalanced in respect to particular elements	Directly used for needs of mineral nutrition, increase plant growth and development	Do not meet optimum needs at low level of application. Certain elements can be at toxic level
Organic matter	Relatively high	Indirect benefit from improving soil properties, long-term effect from acting as nutrients for microorganisms	Decrease in plant availability of certain minerals
Biologically active substances	Highly variable, usually high	Promote plant growth, improve uptake of minerals, induce resistance against pests and diseases	Positive effect will be seen only at optimum level of mineral supply. Include growth inhibitory substances
Microorganisms	Highly variable, usually high	Promote availability of mineral nutrients through mineralization and solubilization. Release biologically active substances	Can contain potentially harmful microorganisms

and development will be promoted due to the increasing doses of plant-available mineral nutrients with the application of vermicompost. Consequently, any amount of vermicompost in relatively poor soil will benefit plant growth. This is especially important in organic agriculture, where organically derived fertilizers with a relatively high degree of mineralization are a valuable choice for increasing plant productivity. However, it is necessary to note that a special care needs to be taken to balance mineral nutrient content in feeding material for earthworms to better address plant needs for essential elements. Usually, vermicomposts are relatively rich in Ca, Mg, Zn, and B and deficient in N, S, Fe, Mn, Cu, and Mo, while P and K can reach extremely high levels (Karlsons et al. 2015). In addition, Na and Cl concentration can be high, especially, if composted livestock manure has been used as a feed for earthworms.

In conditions of optimal soil mineral nutrient availability, high doses of vermicompost might even lead to toxicity of some elements. Consequently, a direct beneficial effect of vermicompost application can be related to (1) high content of hormonelike substances promoting plant growth and development and (2) protection against pests and pathogens. Irrespective of original soil mineral nutrient content, high organic matter and occurrence of microorganisms in vermicompost will promote renovation of soil fertility.

While plant hormonelike activity in compost and vermicompost preparations is a well-known phenomenon (Krishnamoorthy and Vajranabhaiah 1986; Tomati et al. 1988), no attempts have been made to quantify this effect of plants. Recently, we used two different approaches to assess plant growth-affecting activity of organic fertilizers (Ievinsh 2011; Grantina-Ievina et al. 2013, 2014a; Karlsons et al. 2015). The first approach includes measuring an effect of water extract from fertilizers on seed germination and growth of etiolated vegetable seedlings. Four vegetable crop species with a relatively wide range of physiological responses against vermicompost application were selected for the test including beetroot (*Beta vulgaris* L.), Swedish turnip (*Brassica napus* var. *napobrassica* L.), carrot (*Daucus carota* L.), and tomato (*Lycopersicon esculentum* L.). Seed samples were imbibed in water or vermicompost extract at various concentrations and germinated in darkness in the presence or absence of the respective test solution. After 6 days, the hypocotyl height and radicle length of the seedlings were measured, and a degree of stimulation vs. inhibition was calculated. Possible effect of soluble mineral nutrients on plant growth was eliminated by using a second control with mineral nutrient solution at concentration identical to that in vermicompost extract. The method revealed significant differences in plant growth-affecting activity between different organic waste-derived compost and vermicompost samples (Grantina-Ievina et al. 2013). In particular, the highest growth-promoting activity was found for cow manure vermicompost stored wet for 1 year at 4 °C, while storage of the same preparation dry for 1 year at room temperature significantly decreased growth-promoting activity and increased growth-inhibiting activity. Also, plant growth-promoting activity significantly increased when composted sewage sludge were vermicomposted for a short or further for a relatively long period of time.

The second approach allowed to eliminate possible mineral nutrient effects during plant cultivation studies in controlled conditions with organic fertilizer as a substrate amendment (Grantina-Ievina et al. 2014a; Karlsons et al. 2015). The experimental setup allowed to discriminate whether changes in plant growth and development resulted from plant growth-affecting activity or were related to changes in mineral nutrient supply. This was achieved by using two types of control, e.g., pure quartz sand and quartz sand with optimum level of mineral nutrients added. Treatment with increasing doses of organic fertilizers was performed both in the case of pure sand and mineral-enriched sand. It was shown that even 10 % substrate substitution treatment with vermicompost at optimum mineral nutrient conditions resulted in 90 and 98 % increase of fresh and dry mass of winter rye (*Secale cereale* L.) plants (Karlsons et al. 2015). Moreover, further increase of substrate substitution rate with vermicompost (30 and 50 %) resulted in a near-linear concentration-dependent increase in both fresh and dry mass accumulations of rye plants. In consequence, it was concluded that in conditions of optimal soil mineral nutrient availability, a beneficial effect of vermicompost application results mainly from plant growth-promoting activity, while in nutrient-poor soils increase in plant-available minerals due to vermicompost treatment is the most important aspect.

18.3.2 Microbiological Quality of Vermicompost

The wide variety of organic waste (plant residues, animal manure, activated sludge from wastewater treatment plants, etc.) available as feedstock in vermicomposting represents a rich source of microbial diversity. It is reported that vermicompost can significantly increase the amount of plant growth-promoting (free-living nitrogen fixers, nitrifying bacteria, phosphate solubilizers, silicate solubilizers, and fluorescent pseudomonads) and plant disease-protective microorganisms, such as *Trichoderma* spp. fungi in comparison to the initial substrate (coconut leaves with cow manure) used for vermicomposting (Gopal et al. 2009). The application of vermicompost has been used in an investigation in India to increase the level of potentially favorable soil microorganisms such as nitrogen fixers and mycorrhizal fungi (Kale et al. 1992). It has been shown in previous studies that the addition of pig manure and food waste vermicompost significantly increased the microbial activity in commercial substrates (Atiyeh et al. 2000, 2001). Based on molecular analysis, it was found that microbial diversity and species composition of vermicomposts, prepared from mixed organic materials, mainly green plant parts, cattle manure, and agricultural plant waste, were similar to those of vermicompost extracts produced from them. For example, the saprophytic bacteria, *Sphingobacterium* and *Actinomyces*, and ammonium-oxidizing bacteria, *Nitrosovibrio* and *Nitrosospira*, were found in both vermicompost and subsequent extracts (Fritz et al. 2012).

Evidently, vermicompost-associated microorganisms can affect humans during processing; therefore, vermicompost handling needs to be conducted similarly as in conventional composting (Deportes et al. 1995). For example, in a study in Italy of fungal populations of vermicompost produced from 70 % dung (from cows, poultry, and various zoo animals) and 30 % plant debris from various sources, it was found that the fungal populations were dominated by two species: *Pseudallescheria boydii* and *Aspergillus fumigatus* (Anastasi et al. 2005). Both species are potential human and animal pathogens and have been found also in vermicompost samples produced in Latvia from various substrates—cow manure, cow manure with tree leaves, sewage sludge and starchless potato pulp, and composted grass (Grantina-Ievina et al. 2013).

It has been shown that the level of artificially inoculated potentially harmful microorganisms such as *Escherichia coli*, *Enterococcus* spp., and *Salmonella* spp. is significantly reduced due to the activity of earthworms already after 6 days of vermicomposting biosolids from municipal plants (Eastman et al. 2001). Selective reduction of pathogenic bacteria was observed during the vermicomposting of cow manure: the level of fecal enterococci, fecal coliforms, and *Escherichia coli* was reduced, but the level of *Clostridium*, total coliforms, and enterobacteria remained unchanged (Aira et al. 2011). The indicators of fecal contamination such as bacteria *E. coli* and enterococci have been detected in composted sewage sludge and in two consecutive immature vermicompost samples, but in mature vermicompost only *E. coli* was present (Grantina-Ievina et al. 2013). There is also some evidence that the level of potentially pathogenic fungi may remain unchanged during vermicomposting (Beffa et al. 1998) or even increases (Grantina-Ievina et al. 2013). Nevertheless, it has been demonstrated in several investigations that water extracts from vermicompost possess antifungal activity. For example, it is reported that aqueous extracts of air-dried vermicompost inhibited spore germination of several fungi from *Alternaria*, *Curvularia*, and *Helminthosporium* genera and the development of powdery mildews on balsam and pea in India (Singh et al. 2003). In another study, water extracts of vermicompost that was produced from paper sludge and dairy sludge inhibited spore germination of *Fusarium moniliforme* in vitro, but spore germination of such plant pathogens as *Rhizoctonia solani*, *Colletotrichum coccodes*, *Pythium ultimum*, and *Phytophthora capsici* was not reduced (Yasir et al. 2009). Water extracts from vermicomposts produced from cow manure, cow manure with tree leaves, sewage sludge and starchless potato pulp, and composted grass have shown antifungal activity in vitro against fungi from genera *Pseudeurotium*, *Beauveria*, *Nectria*, and *Fusarium* (Grantina-Ievina et al. 2014b).

Much research has been conducted with general bacterial populations, and it is known that particular production conditions (feedstock, time and method of vermicomposting) result in similar species composition of bacterial populations of vermicompost samples if the same earthworm species is used. For example, the average similarity coefficient among various products was nearly 80 % when estimated by comparable methods (Fernández-Gómez et al. 2012).

18.3.3 Case Study: The Impact of Vermicompost on Soil Microorganisms and Potato Yield

The second case study is about the use of vermicompost in organic starch potato cultivation. In the first growing season (2012), the vermicompost produced from composted grass and starchless potato pulp was amended in the amount of 0, 4, 6, 8, 10, and 12 tons/ha in field experiment. The development and severity of the late blight caused by *Phytophthora infestans* were assessed. It was estimated that vermicompost amendments did not reduce the potato late blight infection as it was expected, but in contrary, it was significantly increased (Table 18.2). It can be explained by observed encouraged growth of potato foliage that resulted in more favorable conditions and microclimate for the development of potato late blight infection. The impact of plant density to the potato late blight infection has been described (Hospers-Brands et al. 2008). Nevertheless, the vermicompost increased the potato yield. For example, application of 12 tons/ha of the vermicompost increased potato and starch yields by 15 % and 10 %, respectively, in the first growing season (unpublished data). In the second year of field experiments (2013), granulated form of vermicompost from starchless potato pulp and composted grass was used in the amount of 0, 1, 2, and 3 tons/ha. The largest amount of the granules

Table 18.2 Incidence of potato late blight pathogen *Phytophthora infestans* Deb. infection (%)

	Time of assessment		
Amount of vermicompost (tons/ha)	24 July 2012	31 July 2012	09 August 2012
0	0	7.9	33.4
4	0	9.9	33.8
6	0	10.9	43.8
8	0	12.1	52.8
10	0	13.1	63.8
12	0	12.8	68.1

increased the potato and starch yields by 15–30 % depending on the field (unpublished data). During the growing season, the plant response to the vermicompost amendments was monitored in the terms of photosynthetic activity and leaf chlorophyll content, and in particular measurement times, significant changes of these parameters were detected.

The impact of the vermicompost on several groups of soil microorganisms (total bacterial population, number of Actinobacteria, and filamentous fungi) was assessed. It was concluded that vermicompost amendments did not significantly change the abundance of these microorganisms, while the species spectrum of filamentous fungi was altered. For example, the application of 1 tons/ha significantly increased the amount of plant growth-promoting filamentous fungi, such as *Mortierella* and *Trichoderma* spp. (unpublished data).

18.4 Conclusions

It is expected that organic farming with the application of green manure or vermicompost would result in high biodiversity of soil organisms and plant growth promotion. On average, significantly higher numbers of all groups of analyzed cultivable microorganisms were observed in organic agriculture fields in comparison to conventional fields, e.g., total bacterial population had increased by 70 %, Actinobacteria by 290 %, and cultivable filamentous fungi by 110 %. Results obtained by molecular methods regarding fungal diversity did not show such an increase.

In contrast to the soil microbiological indicators, controversial results about plant health, in terms of disease suppressiveness, have been obtained. Our studies raise particular concerns about the vermicompost. Definitely, the unique nature of organic amendments in each case must be taken into account. Further studies are needed to explain the impact of green manure and vermicompost on the plant health.

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