EFFECT OF VARIOUS NITROGEN SOURCES ON SOIL BIOMASS AND OTHER SOIL BIOLOGICAL PARAMETERS

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Abstract:

Pot experiment was run out to study the effects of various nitrogen sources on microbial biomass (C and N) and other soil biological properties in Kovarvany brown forest and meadow chernozem soil types incubated at temperature 25±3°C. Pots were filled up with 2 kg of soil amended with sewage sludge at the levels of 0, 12.5, 25, or 50% (w/w) in comparison with soil amended with ammonium nitrate at 0, 11.5, 23 and 46 mg/kg soil for eight weeks and untreated control. Soil aerobic heterotroph bacterial enumeration, fungal population, soil basal respiration, total soil organic carbon, dehydrogenase and FDA potential activities were investigated. Results indicated that the addition of sludge or NH_4NO_3 caused changes in the microbial population sizes which increased significantly with the increases of the organic fertilizer (sewage sludge) rates more than the inorganic fertilizer (NH_4NO_3). In addition, sewage sludge had a stimulatory effect on the soil enzymatic potential activity and increases the total organic carbon. The highest number of fungi observed in meadow chernozem soil even treated with NH_4NO_3. It was found that Kovarvany brown forest soil had lower soil biomass and biological properties when treated with NH_4NO_3. Moreover, meadow chernozem soil had higher biological properties when treated with sewage sludge than NH_4NO_3 over the Kovarvany brown forest soil. These results suggest that the positive association between organic fertilizer amendment and soil quality where enhance both microbial and enzymatic activities. However, the results indicated that soil enzyme activities have been proposed as appropriate indicators because of their intimate relationship to soil biology, ease of measurements, and rapid response to a small change in soil management.

Keywords:

Nitrogen sources - soil biomass - biological parameters

INTRODUCTION

Soil properties based on biological and biochemical activities, especially those involved in energy flow and nutrient recycling; have been shown to respond to small changes in soil conditions, thus providing information sensitive alterations of soil quality. Quantitative and representative recovery of microbial activities from environmental samples is essential in understanding ecosystem function. Soil microorganisms are essential to the environment due to their role in forming soil structure, cycling mineral compounds, decomposing organic materials, promoting/suppressing plant growth, and various soil biological-physical-chemical processes [1]. They play crucial roles in biogeochemical cycling and ecosystem functioning [2], and thus influence soil fertility and ecosystem stability directly. However, soil microorganisms are sensitive to changes in land-use patterns, tillage practices, and management treatments [3], for instance, fertilization may influence the population, composition, and function of soil microorganisms. Therefore, soil microbiological and biochemical properties such as microbial biomass, community composition, metabolic activity, functional diversity, and various enzymatic activities are often measured to provide immediate and accurate information about small changes in soils.

Major challenges for combined use of organic and mineral nutrient sources in agriculture include variable type and quality of the N sources, their limited availability, timing of their relative application and the proportions at which the two should be combined. Application of organic sources usually leads to increased crop yields [4]. The differential yield responses following organic source application have been attributed mainly to differences in organic source quality and soil fertility status [5]. One of the pathways to lose soil C in a row cropping system as a result of management practices (i.e., tillage, N fertilization, etc.) is the emission of CO_2 from soil. This soil C loss mechanism as affected by management practices can be determined and quantified by either measuring CO_2 emission or through other indicators such as change in soil microbial biomass C or change in soil C fractions. Generally, sources of CO_2 from a soil system can be attributed to biological and chemical activity within the soil. Soil respiration involves organisms metabolizing substrates producing CO_2 within the soil matrix [6]. Carbon dioxide loss from soil can also be associated with microbial decomposition of organic matter and root respiration [7]. The mechanism of soil CO_2 emission to the atmosphere, however, involves the movement of CO_2 through soil pores, and release from the soil system can be measured at the soil surface [8]. The combined application of organic sources and fertilizers is increasingly gaining recognition as one of the appropriate ways of addressing soil fertility depletion, especially in low-external input systems in sub-Saharan Africa and forms an integral part of integrated soil fertility management [9, 10]. Greater yield benefits can be achieved following the combined application of organic resources and fertilizers compared to either resource applied alone [11]. Tan et al. [12] mentioned that soil enzymes are known to be involved in nutrient cycling, and as such, their activities can be used as potential indicators of nutrient cycling processes. In addition, soil enzymes are specific for the types of chemical reactions in which they participate. Dehydrogenase plays an important role in the initial oxidation of soil organic matter and occurs only in viable cells; therefore, it is believed that dehydrogenase is an intracellular enzyme involved in microbial respiratory processes [13].

MATERIALS AND METHODS

Soil characterization and sampling

Bulk soil samples were obtained from farmland surface layer (0-250 mm) that, for many years, was not treated with pesticides. The laboratory pot experiments were laid out with two soil types: kovarvany brown forest and meadow chernozem collected from of University of Debrecen, Centre of Agricultural Sciences, Operating area of brown forest soil of Research Centre of Nyíregyháza and Institute of Cereal Research, Szeged, respectively, Hungary. Some selected physico-chemical parameters of the two soil types are given in Table 1.

Table 1. Some physico-chemical properties of investigated soil types

Parameters	**Soil samples**	
	Kovarvany Brown Forest	**Meadow Chernozem**
Topsoil profile	Sandy loam	Clay loam
$pH_{(KCl)}$	5.78	6.20
Humus content [%]	2.54	3.55
NO_3-N [mg/kg]	2.30	3.90
NH_4-N [mg/kg]	5.60	4.50
Ca [mg/kg]	893	1136
Mg [mg/kg]	214	257

Na [mg/kg]	64	53
P_2O_5 [mg/kg]	318	378
K_2O [mg/kg]	412	428
Zn [mg/kg]	1.70	1.10
Cu [mg/kg]	1.40	2.4
Mn [mg/kg]	55	61
Fe [mg/kg]	945	1094
Cd [mg/kg]	1.70	1.02
Pb [mg/kg]	1.30	0.96

Organic N source: Sewage sludge

Sewage sludge sample was obtained from the municipal water treatment plant, Nyíregyháza, which is an activated sludge treatment plant. Some selected physico-chemical parameters of the municipal sewage sludge are given in Table 2.

Table 2. Some physico-chemical properties of investigated soil types

Parameters	**Sewage sludge characteristics**
$pH_{(KCl)}$	6.1
Dry matter content %	63
Organic matter %	29
Total N content, mg/kg	5600
Ca, mg/kg	24150
Mg, mg/kg	2224
Na, mg/kg	791
P_2O_5, mg/kg	6040
K_2O, mg/kg	1166
Zn, mg/kg	91
Cu, mg/kg	45.6
Mn, mg/kg	360
Fe, mg/kg	6757
Cd, mg/kg	1.0
Cr, mg/kg	15.7
Ni, mg/kg	10.2
Pb, mg/kg	56.3
Hg, mg/kg	1.88
As, mg/kg	8.08

Inorganic N source:

Ammonium nitrate (NH_4NO_3)

Soil amendment with N sources

Fresh soil samples were air dried, sieved through a 4 mm sieve and placed in polyethylene bags. Soil samples were stored at 4°C until used. Before use, soil samples were kept for air dry environment at room temperature (approximately 28°C) for 24 hours. The two N sources were added to soil at 4 rates. Different N rates were prepared by dilute the inorganic N source ammonium nitrate with sterile distilled water on the basis of the active ingredient rate added to the agricultural farm as fertilizer to reach 0, 11.5, 23 and 46 mg/kg soil and the municipal sewage sludge was applied to the soil samples on the basis of weight by weight at the levels of 0, 12.5, 25, or 50% (w/w). For both, soil samples well mixed with the two N sources. The addition of the N sources to the soil was done as a part of the moisture required to adjust the soil to 45% of their water holding capacity. The inorganic N solution was sprayed onto the soil surfaces by means of a syringe that dispensed very small droplets. Pots of 2 kg of untreated control and N amended soil samples were incubated at 25±3°C for 8 week. Loss of water by evaporation was compensated daily to avoid dryness. Pot experiment was run out to study the effects of various nitrogen sources on microbial biomass (C and N) and other soil biological properties in Kovarvany brown forest and meadow chernozem soil types incubated at temperature 25±3°C. Pots were filled up with 2 kg of soil amended with sewage sludges at the levels of 0, 12.5, 25, or 50% (w/w) in comparison with soil amended with ammonium nitrate at 0, 11.5, 23 and 46 mg/kg soil and control soil of 0 amendment for eight weeks.

Soil microbial biomass C and N measurements

Microbial biomass C, and biomass N were estimated by the fumigation-extraction method according to Brookes et al. [14] and Vance et al. [15]. The factors used to convert the extracted organic C and N to MBC and MBN were 0.38 and 0.45 respectively. Microbial biomass C/N was calculated on an oven dry soil weight basis.

Soil basal respiration

Soil basal respiration was determined using the sealed incubation-alkali absorption method as described by Anderson [6], and CO_2 evolution was measured by titration of unconsumed 1 M NaOH in the CO_2 traps with 1 M HCl (CO_3^{2-} was precipitated with Ba^{2+} before titration).

Dynamics of Organic Carbon

Total organic carbon (TOC) was analyzed by dichromate ($K_2Cr_2O_7$) oxidation and titration with ferrous ammonium sulphate according to the method of Walkley and Black [16].

Determination of Enzymatic Activities

Fluorescein diacetate (FDA) hydrolyzing activity of the soil sub-samples were determined by measuring the released fluorescein at 490 nm according to Schnürer and Rosswall [17]. Dehydrogenase activity was determined by the method of García et al. [18].

Enumeration of Some Microbial Population Densities

The enumeration of soil microbiota was done by the serial dilution spread-plate technique at the start and end of incubation period of pot experiment and expressed in the form of total colony forming units (CFU) of aerobic heterotroph bacterial and fungal populations in nutrient agar (NA) with cycloheximide (100 µg/ml) as antifungal growth inhibitor and Difco Czapek-Dox agar and potato dextrose agar (PDA) media were used for fungal population, respectively. Rose Bengal (RB) was added to Czapek-Dox agar and PDA at 65 ppm as a bacteriostatic agent.

RESULTS AND DISCUSSION

Determination of MBC and MBN

The microbial biomass C and N content depended on the C and N amendments. As shown by the evaluation of the particular treatments, the highest content of microbial biomass was determined at the application of wastewater sludge in both soil types (Figures 1 and 2). Microbial biomass C and N were higher in the two soil types when treated by organic than inorganic fertilizers in comparison with the untreated control soil samples. Figure 1 illustrates the influences of both fertilizers on the MBC. It shows that by increasing the rate of application, the MBC increases and the MBC due to the combination of organic (25%) and inorganic (0.5x) treatment lay between the two maximum MBC of obtained at field applied dose (x) and 50% (w/w).

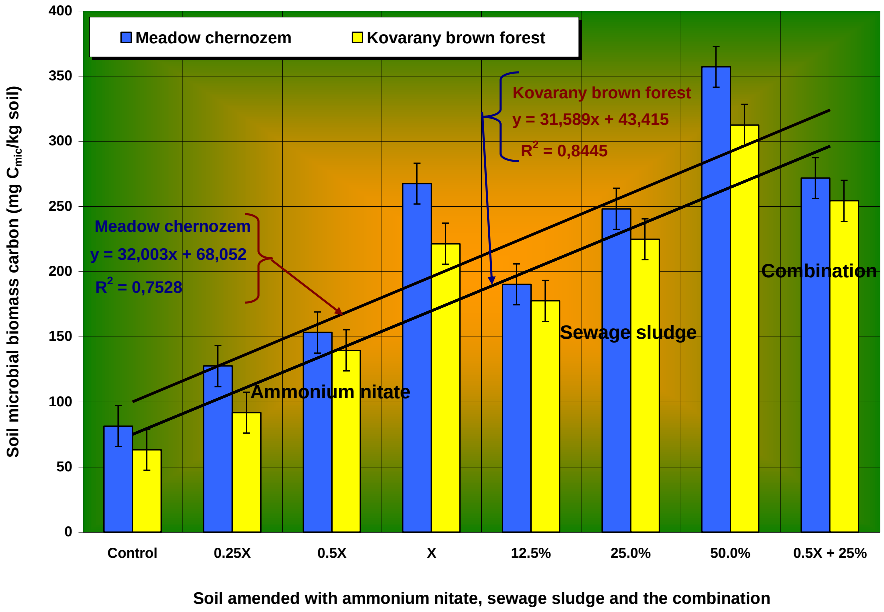

Figure 1. Effects of nitrogen sources on microbial biomass carbon in different soil types

Meadow chernozem soil type had higher MBC than in kovarvany brown forest soil types. Figure 2 shows similar pattern was found also in case of microbial biomass nitrogen (MBN), the highest MBN values were reached at the highest doses of ammonium nitrate and sewage sludge applied to the two soil types. Also, meadow chernozem soil type had higher MBC than in kovarvany brown forest soil types. Our results were agreed with Coote and Ramsey [19] who stated that N fertilizers had an indirect positive influence on soil microbial biomass. Higher content of MBN and higher content of MBC were determined for the organic and inorganic combination application. This finding corresponds to conclusions published by Ocio et al. [20], who measured about 18% higher content of microbial biomass N over the control. It was found that the content of microbial biomass depended on the treatment, soil type, climatic conditions of incubation and the time too. Higher contents were found in treatments with organic fertilizers. The highest contents of MBC and MBN were estimated in the pots treated with sewage sludge. High amounts of organic inputs often result in high microbial biomass [21]. García-Gil et al. [22] found MBC to be 33% higher in soils receiving municipal solid waste compost at the annual rate of 80 t/ha than at 20 t/ha. A positive influence of the manure application on the microbial biomass content was observed.

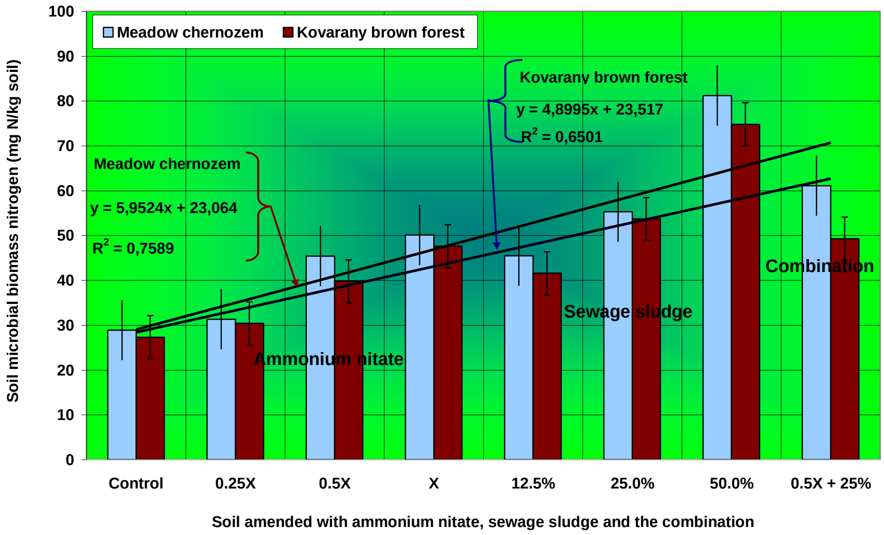

Figure 2. Effects of nitrogen sources on microbial biomass nitrogen in different soil types

The highest contents of MBC and N in pots with organic fertilizers were found in samples collected from the 2 soil types. Our results are in agreement with Ghoshal and Singh [23] recorded the maximum increase in the microbial biomass, due to the inputs of the manure + fertilizer treatment followed, in decreasing order, by manure alone and fertilizer alone.

A high content of easily decomposable organic C can lead to fast growth of soil microbes, likely resulting in a higher microbial biomass and its activity. Chowdhury et al. [24] observed that manure compost with high easily decomposable C was more effective than sawdust and rice husk composts in enhancing soil MBC. Flieβbach and Mäder [21] described that mineral fertilization had a distinct effect on crop yield, but no positive effect on microbial biomass. However, the addition of N + sludge treatment caused an increase in the content of MBN compared to N-fertilizers treatment.

Effects of N sources on CO_2-release

In both soil types, it was found that low and high levels of inorganic fertilizer did not improve the CO_2-release significantly with the untreated control soil samples. While the applications of 25% and 50% organic and combined fertilizers significantly improved the production of CO_2. The amount of CO_2-release from Kovarvany brown forest soil was lower than the amount released from meadow chernozem soil (Figure 3). Several biological parameters, e.g., microbial biomass, enzymatic activities and CO_2-release have been used to define the status and sustainable development of productivity in soils, and are used as bioindicators for soil quality and health in environmental soil monitoring. Microbial biomass C is measured to give an indication of the response of soil microbial activities to management, environmental change, site disturbance, and soil pollution [25]. Soil respiration, as measured by the net heterotrophic production of CO_2, is an important measure of aerobic microbial activity and carbon flux through terrestrial ecosystems [26]. The CO_2 produced from the soil results from the mineralization of organic matter, a process in soil microflora play a dominant role [27].

Effects of N sources on total organic carbon

Figure 4 illustrates that the total organic carbon (TOC) increased with increasing the concentrations of the inorganic as well as the organic fertilizers. The results indicated that 50% soil amendment with sewage sludge improved the percentage of total organic carbon in both soil types. Total organic carbon in Kovarvany brown forest soil was lower than the percentage of TOC in meadow chernozem soil. The application of 23 g ammonium nitrate/kg soil in combination with 25% of sewage sludge increased the percentage of TOC in both soil types significantly compared with control. Statistically, the correlation the application of N fertilizers and total organic carbon were moderately high. The correlation (R^2) was higher in case of meadow chernozem (0.7234) than in the case of Kovarvany brown forest soil which was 0.7159.

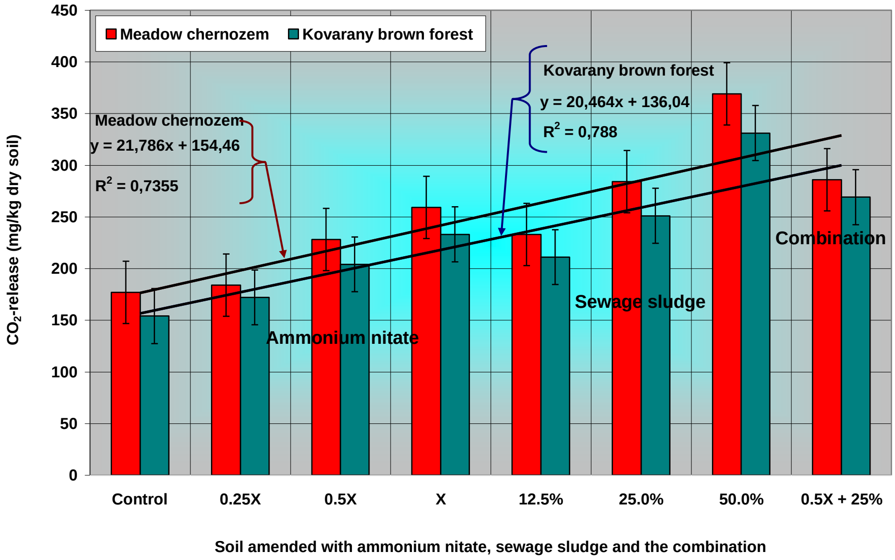

Figure 3. Influences of various nitrogen sources treatments on soil basal respiration in various soil types

Nutrient cycling and the associated yield benefits derived from combining organic resources and fertilizers are dependent on a number of factors including climate, bio-physico-chemical soil environment and organic resource quality and there are intricate interactions among these factors [28].

Tian et al. [29] observed increases in rate of decomposition and nutrient release with increase in organic resource quality in wetter climates but in drier climates decomposition and nutrient release was faster with low quality organic resources than high quality organic resources. Low quality organic resources have a mulching effect that influence soil microclimate and thus enhance their decomposition [29]. While the combined addition of intermediate quality organic resources with N fertilizers or the sole addition of high quality organic resources can enhance nutrient cycling and increase crop yields, their effects on soil organic C (SOC) build-up may be negative.

The addition of fertilizers with intermediate quality organic resources may increase organic resource decomposition [30] and thus may result in reduced SOC stabilization compared to organic resource added alone. The addition of intermediate and low quality organic resources may result in greater SOC concentrations than high quality organic resources [31]. However, recent studies have shown no long-term effects of organic resource quality on SOC dynamics [32].

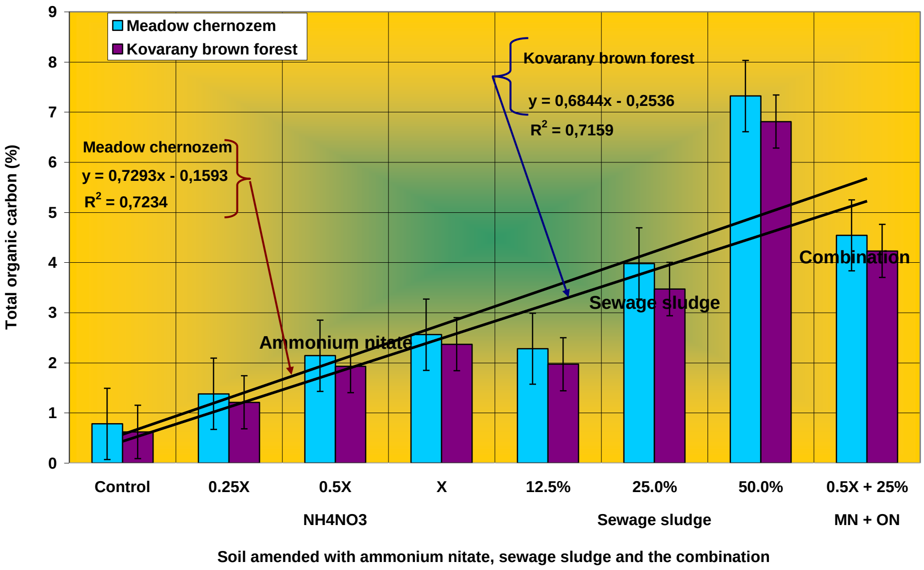

Figure 4. Impacts of different doses of nitrogen sources applied to two soil types on total organic carbon

Effects of N sources on potential enzymatic activities

The increase of enzymatic activities with the sewage sludge rate is probably due to the addition of labile C supplied by this organic fertilizer, indicating that the microbial biomass is active and potentially mineralized organic matter. Soil enzyme activities are used as indicators of microbial activity and react quickly to environment change. Figures 5 and 6 show the evolution of two enzymatic activities measured at the end of the experimental period.

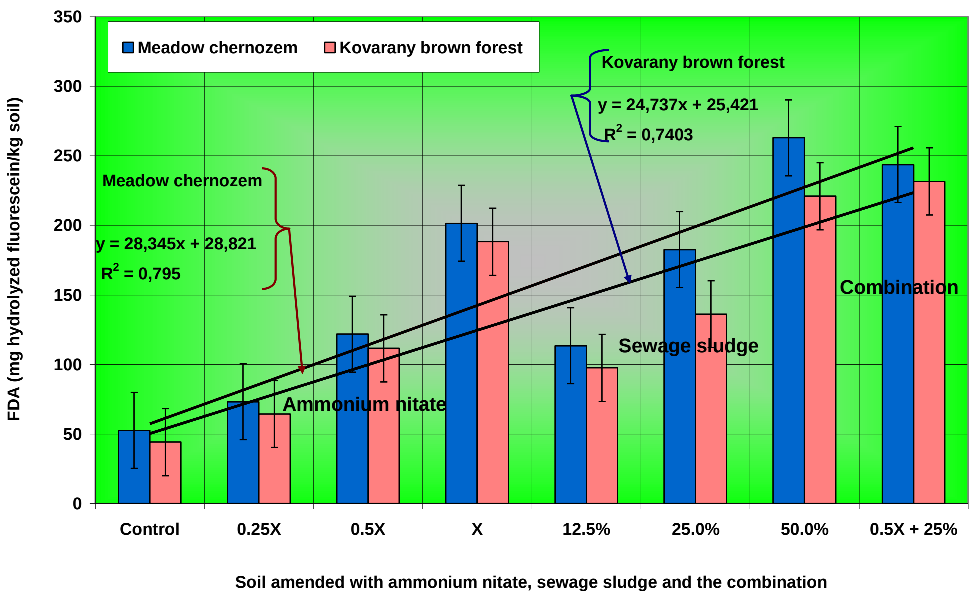

Figure 5. Effects of nitrogen sources on FDA activity in different soil types

The results showed that elevated organic fertilizer mixing rate differently impacted on soil potential enzyme activities. Increases of potential activities of FDA (Figure 5) and dehydrogenase (Figure 6) under addition of organic fertilizer occurred with increasing the application rate. These increases were more significant in soil amended with organic fertilizer than with inorganic fertilizer compared to control. The results showed that the potential activity of FDA was higher in soil samples amended with a mixture of organic and inorganic fertilizers than in inorganic fertilizer alone, and there was no significant difference with the sewage sludge application at 50%. Dehydrogenase potential activity has been proposed as an indicator of the total metabolic activity of soil microorganisms [33]. In our experiment, this potential enzymatic activity increased by the addition of sewage sludge to the both soil types and remained stable throughout the subsequent incubation time (Figure 6).

It is important to mention that the dehydrogenase potential activity increased with the addition of more organic fertilizer than inorganic ammonium nitrate fertilizer. Meanwhile, the combination of 25% organic and 23 g inorganic fertilizers increased the potential activity of dehydrogenase than when the soil treated with 46 g ammonium nitrate or 25% sewage sludge separately. Also, it was found that the potential activity of dehydrogenase in Kovarvany brown forest soil was lower than the potential activity in meadow chernozem soil.

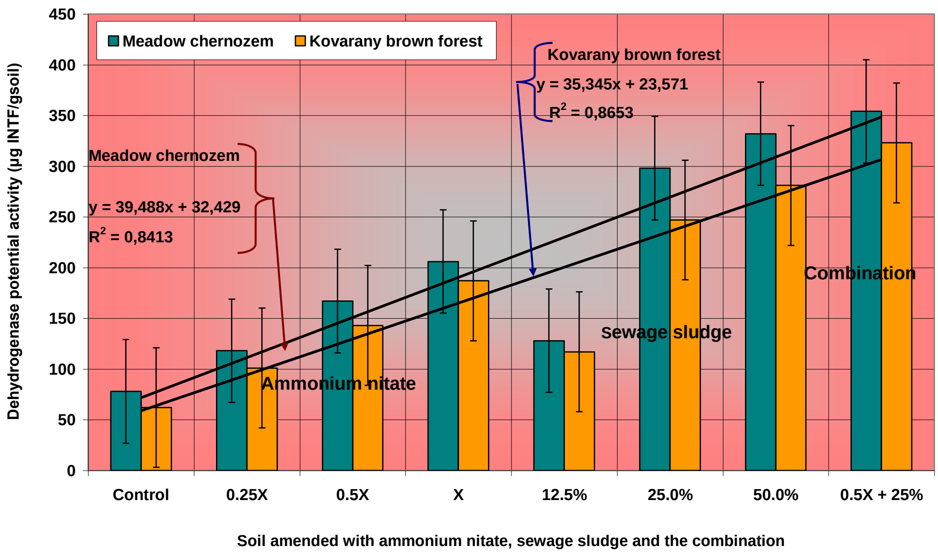

Figure 6. Effects of nitrogen sources applied to different soil types on dehydrogenase potential activity

Soil enzymatic activities, which can be used as another index of microbial functional diversity [34], have been suggested as potential sensitive indicators to reveal changes of soil quality due to soil management and to monitor soil microorganism activity related to soil nutrient transformation [35]. For example, invertase drives C cycling by catalyzing the hydrolysis of sucrose. Thus, testing the activity of soil invertase may be useful for evaluating soil capability of decomposing complex organic compounds into subunits that can be assimilated by microorganisms or plants, in other words, such enzymatic indices would integrate other chemical, physical, and biological characteristics and could monitor the effects of agricultural management on soil productivity [36]. Organic and inorganic fertilizers are used primarily to increase nutrient availability to plants [37]. The correlations between the potential activities of dehydrogenase in both soil types Kovarvany brown forest and meadow chernozem (R^2 = 0.8923 and R^2 = 0.8892, respectively) were higher compared with the correlations in case of TOC and FDA (R^2 = 0.7403 and R^2 = 0.7950, respectively).

Effects of N sources on microbial contents

Figures 7 and 8 illustrate the enumeration of aerobic heterotroph bacterial and fungal population in both tested soil types, respectively. Results illustrated that soil amended with organic fertilizer had more aerobic heterotroph bacterial population than the soil treated with inorganic fertilizer. Maximal bacterial population was recorded when the soil treated with 50% sewage sludge followed by the fertilization with the combination of organic and inorganic fertilizer. Also, it was found that the fertilization with inorganic ammonium nitrate did not improve the bacterial population. Statistically, the correlations in case of Kovarvany brown forest and meadow chernozem were (R^2 = 0.8923 and R^2 = 0.8892, respectively).

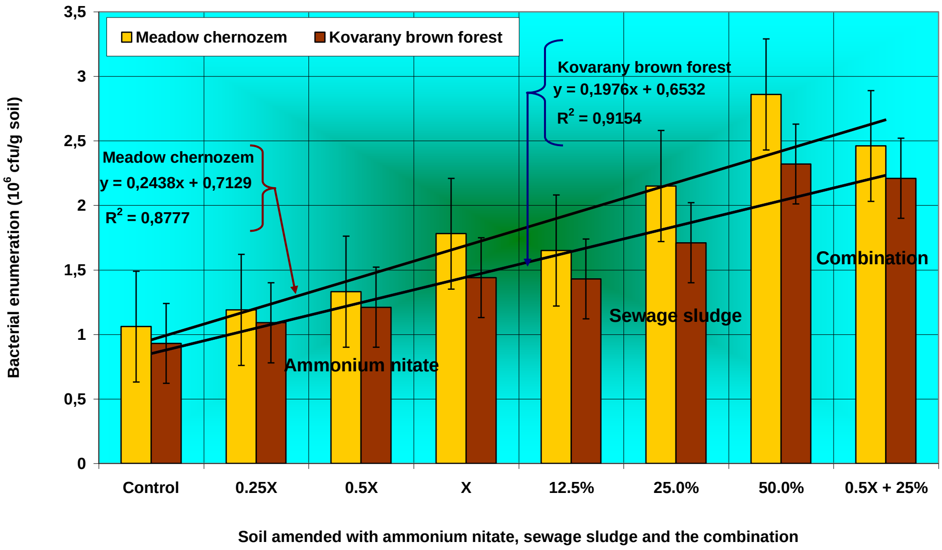

Figure 7. Effects of nitrogen sources on aerobic heterotroph bacterial population in different soil types

It has long been recognized that appropriate community population, abundant diversity, and high activity of microorganisms are significant factors to maintain the sustainability and productivity of terrestrial ecosystems [38]. Similarly, the fungal population was depending on the amount of carbon and nitrogen in the soil. Figure 8 shows the dynamic of fungal population density in the soil amended with inorganic or organic or in a combination of both of them. It was found that sewage sludge at any concentration improved the population density of fungi in both soil types, but Kovarvany brown forest had lower population than it was found in meadow chernozem soil in any case. Also, the combination of 25% of sewage sludge and 23 g ammonium nitrate increased the fungal population more than the application of inorganic fertilizer at recommended field dose (46 kg/ha). Statistically, with the comparison with the population of fungi at control soil types, it was found that there was no significant difference between the population of fungi and the application of inorganic fertilizer at any level, but it was significantly when both soil types are treated with different doses of sewage sludge.

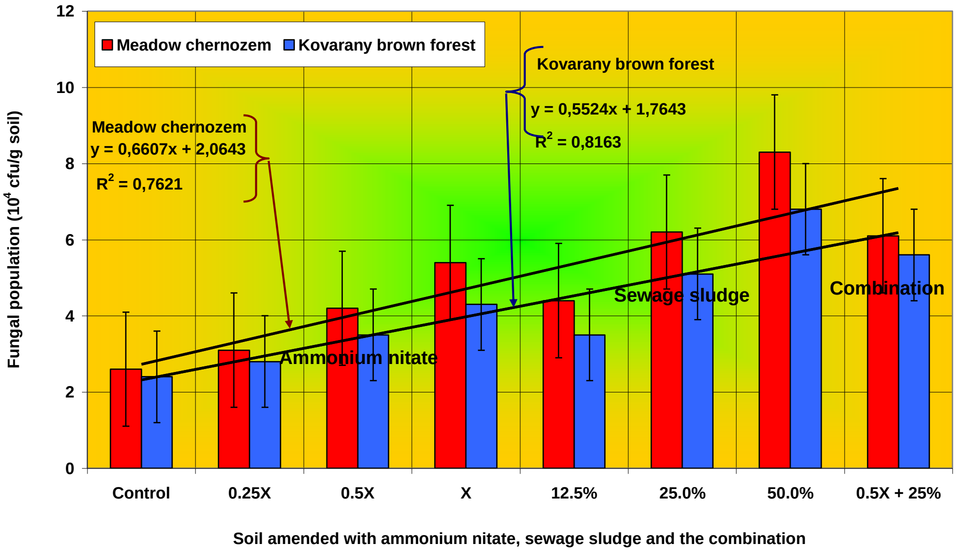

Figure 8. Effects of nitrogen sources on fungal population density in different soil types

However, soil microorganisms are sensitive to changes in land-use patterns, tillage practices, and management treatments [3], for instance, fertilization may influence the population, composition, and function of soil microorganisms. Therefore, soil microbiological and biochemical properties such as microbial biomass, community composition, metabolic activity, functional diversity, and various enzymatic activities are often measured to provide immediate and accurate information about small changes in soils.

CONCLUSION

An increase in the size of the soil microbial biomass is considered essential for the improvement of soil fertility. The results of the present study suggested that the positive correlation between organic fertilizer amendment and soil quality where enhance both microbial and enzymatic activities. However, the results indicated that soil enzyme activities have been proposed as appropriate indicators because of their intimate relationship to soil biology, ease of measurements, and rapid response to a small change in soil management. Finally, the best suggestion is the amendment of agricultural soil with a combination of organic and inorganic fertilizer to reduce the soil pollution and increase the soil sustainability.

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