"In-vivo" and "in-vitro" Experiments on the Influence of Compost Preparations and Heavy Metals on Soil Enzymes Activities and Soil Health

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RESEARCH ARTICLE

Abstract

Enriching soils with composts and organic waste material will be necessary because high C soils have strong climate change resilience and guarantee stable yields. Annually ≈1 billion tons of agricultural waste is produced which can be brought back to soils after adequate treatment and can relieve atmosphere from CO2. In a field trial municipal solid waste compost (MSW), sphagnum peat (WT), and black peat (HT) were tested. Basic soil parameters pH, C-content and plant nutrients (K, Mg) are significantly increased by MSW. WT and HT were uninfluential. Similar effects are found for microelements Fe, Mn, Zn, and Cu. MSW increased microelements significantly.

Keywords: compost, glucosidases, heavy metals, phosphatases, protease

INTRODUCTION

The environmental and social costs of intensive agricultural production have led to calls for more ecologically based approaches to management (Drinkwater et al. 2017; Schipanski et al., 2016). Ecological management practices shall maintain crop productivity while producing a range of ecosystem services both on-farm and to society in general (Power, 2010; Robertson et al., 2014). At present soil tillage and also soil health moves more and more in the focus of agriculturists as well as environmentalists. Discussions show that one central point is loss of soil organic matter resulting from intensive and/or wrong tillage systems as well as erosion which reduces the fitness of soils and their productivity (Engels, C., et al., 2010). There arises a need to assess soil quality in the course of development of sustainable agricultural and horticultural production systems. The „4 pro mille"-action claims to restore soil carbon by 0.4% annually in order to capture atmospheric CO2 and store it in the soil body (4 per 1000" Initiative, 2022). Similar statements can be found in the latest IPPC report which stresses the role of agriculture including the soils to partly over-come the looming implications of climate change (IPCC Report. 2021). Despite the possible inputs of mineral fertilizers and pesticides, the basic and unique of interest in sustainable soil systems is its capability to cycle and store nutrients and C (Warkentin 1995; Dick 1992). Besides these global efforts as described in the above cited IPCC report, it is necessary to enrich and improve soils with organic amendments in order to capture CO2 and use the positive features of higher soil C contents (Bagnall et al. 2022). In view of the huge amounts of agricultural waste material (straw, by-products of food manufacturing a.s.o.) which sum up to 2 billion tons worldwide it should be possible to recycle a distinct percentage in form of composts in order to improve soils and simultaneously store atmospheric CO2 (Singh et al. 2021).
But then it has also made sure that these materials are save and exert no impacts on the environment and soil health and its productivity (Alvarenga, P. 2015). Particular attention should therefore in this context be paid to the heavy metal load of these materials. Despite some complexing properties of some substances, it cannot be excluded that they are solubilized and may exert negative influences on the complete soil system (Kandeler, F. et al. 1996). Therefore, we planned a field test with a municipal solid waste compost in order to study its influence on soil chemical and biological parameters.

MATERIALS AND METHODS

Test plots
The plots are located in the site “Geisenheim Monrepos” (49.981721 oN; 7.956536 oE, 98 m a.s.l.). Soil is a loess derived loam with an average CaCO3 content of 5%. All plots are planted with shrubs usually used in landscaping.

Treatments
Prior to planting plots received different treatments with soil conditioners and composts:
2. 400 kg/100 m2 black peat (HT)
3. 400 kg/100 m2 sphagnum peat (WT)
4. 2000 kg/100 m2 compost [Municipal Solid Waste] (MSW1)
5. 4000 kg/100 m2 compost [Municipal Solid Waste] (MSW2)
The organic material was worked in the soil with a rotary cultivator to a depth of 20 cm.

All treatments were replicated threefold.

Three years after implementation soil tests and investigations were run.

Soil analysis
In every plot 12 soil samples were randomly taken and thoroughly mixed. Sampling depth was 0-10 cm (LI) and 10-20 cm (LII). Samples were air dried, mortared and sieved (< 2 mm).

 Tested soil chemical parameters: Total-C, pH, P and K according the VDLUFA methods; microelements Fe, Zn, Mn and Cu according to Lindsey and Norvell (1978).

Soil enzyme activities: Neutral and alkaline phosphatase activity (Tabatabai and Bremner, 1969); α-glucosidase and β-glucosidase (Hayano (1973), protease activity (Spier and Ross, 1975).

Data analysis: after testing for homogeneity ONE WAY resp. TWO-WAY ANOVA.

RESULTS AND DISCUSSIONS

Soil chemical parameters
Table 1 presents all basic soil parameters including assimilable plant nutrients for all treatments. Soil pH in the upper layer varies between 7.44 and 7.52. Treatments with WT and HT show significant lower values because these amendments are themselves very acid. In 10-20 cm depth the same pattern is observed. Compost treatments have no influence on pH because of their more or less neutral reaction.

Total C: Application of compost and peat material increases soil carbon significantly. Compost (MC1 and MC2) which is applied in really high rates causes outstanding C contents respectively in LI. No influence can be found in LII for all treatments.

Table 1. Influence of different organic amendments on general soil chemical parameters

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<tbody>
<tr>
<td>pH</td>
<td></td>
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</tr>
<tr>
<td>00-10</td>
<td>7.51 Aa b</td>
<td>7.52 Aa</td>
<td>7.51 Aa</td>
<td>7.46 Ab</td>
<td>7.44 Ab</td>
</tr>
<tr>
<td>10-20</td>
<td>7.50 Aa</td>
<td>7.52 Aa</td>
<td>7.50 Aa</td>
<td>7.44 Ab</td>
<td>7.43 Ab</td>
</tr>
<tr>
<td>Total C (%)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>00-10</td>
<td>0.85 Aa</td>
<td>1.39 Ab</td>
<td>1.94 Ac</td>
<td>1.20 Ad</td>
<td>1.14 Ad</td>
</tr>
<tr>
<td>10-20</td>
<td>0.73 Ba</td>
<td>0.81 Ba</td>
<td>0.90 Ba</td>
<td>0.80 Ba</td>
<td>0.76 Ba</td>
</tr>
<tr>
<td>P_Cal (ppm)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>00-10</td>
<td>337 Aa</td>
<td>358 Aa</td>
<td>325 Aa</td>
<td>346 Aa</td>
<td>351 Aa</td>
</tr>
</tbody>
</table>
Macronutrients (Table 1)
Soil amendments may have a strong influence on nutrients which is mainly dependent on their original nutrient load. Table 1 lists the most important macro-nutrients P, K, and Mg. According to the testing methods slightly soluble and parts of the immobile reservoir are determined. Phosphorus is more or less immobile. The vertical comparison of LI and LII of all treatments do not differ significantly in their P concentration as well as the horizontal comparisons. Potassium behaves different to P. It is significantly lower in LII and the treatments WT and HT. Compost treatments also enrich LII because of their high K load. The vertical comparisons show a significant increase after compost application as well as WT. A clear stratification is found for magnesium. The vertical comparison shows an accumulation in the upper layer and significant higher amount in MC1 and MC2. Normally Mg is higher in deeper layers, but the leaching is probably restricted by the low rainfall rates in that region.

Micronutrients (Table 2)
According to Lindsay and Norvell (1978) the critical levels for Fe, Zn, Mn, and Cu are 4.0-5.0, 0.5-1.0, 1.0-5.0, and 0.1-2.5 ppm respectively.
Iron exceeds in all plots the critical level as well in LI as in LII. Compost application increases Fe-concentrations significantly. Similar statements can be made for zinc. Treatment with compost raises Zn concentrations significantly in LI. Manganese does not increase following the compost treatment. In all plots a significant stratification between LI and LII can be seen.
Copper does not go below the critical level in every plot, but compost application raises the concentrations significantly. There exist also a strict and significant stratification between LI and LII.

Soil enzyme activities
The enzymatic activity (EA) in the soil is mainly of microbial origin, coming from intracellular, cell-associated, or free enzymes. A balanced equilibrium of chemical, physical, and biological (including enzyme activities) components makes up soil health. Evaluation of soil health should include all these components. Healthy soils are essential for the integrity of terrestrial ecosystems to remain intact or to recover from disturbances, such as drought, pest infestation, pollution, and human exploitation including agriculture (Ellert et al. 1997). Soil enzymes play an important role in maintaining soil ecology, chemical properties and soil health. They have key functions in organic matter decomposition and buildup of stable humus matter (Sinsabaugh et al. 1991).
In our investigations α-glucosidase and β-glucosidase, neutral and alkaline phosphatase, and protease (Table 3) were analyzed representative for the soils’ C-, P-, and N-cycle.

For all tested EAs it can be demonstrated that highest values are found in the upper layer (LI) with a strong tendency to a decrease in LII. In all cases the lesser activities are significant on a level p ≤ 0.05. Comparing the EAs horizontally the following results can be demonstrated.

α-glucosidase is significantly highest in MC1 and MC2 in the top layer. Peat based amendments (WT and HT) have no influence. This indicates that the composts contain or re-lease a lot of carbonaceous material, which induces the formation of decaying enzymes.

β-glucosidase activity is quite higher (6-9 times) than α-glucosidase indicating that greater amounts of cellulosic material is released resp. worked in and degraded in the soil. The upper layer LI has highest activities. In LII activities are roughly 50% lower. Nor compost neither peat amendments have an influence on the enzyme activity. It seems that the higher input of organic debris via the root system and leaf litter is responsible for this enzyme distribution.

Neutral and alkaline phosphatases are the types, which will dominate at the prevailing soil pH (Table 1). As stated for glucosidases also both phosphatases show in all treatments a strict and significant stratification with soil depth (Table 3).

Compost doubles (MC2) neutral phosphatase up to fourfold (MC1). Alkaline phosphatase is roughly doubled in both compost treatments.

Protease activity (Table 3) shows also in all treatments a significant decrease from LI to LII. The horizontal comparison shows that MC1 and MC2 have highest protease activities in LI. Activities in WT and HT are lower but significant different from C. In LII exists also an influence of the treatment but not so distinct as in LI. From these results it can be concluded that amendments with higher amounts of degradable organic material may act as a stimulus for soil biological activity.

Analyzing the data set of the different soil enzymes it seems that the C content of the soils is responsible for the varying activities. In general, it can be stated that the activities in LI reach only ≈65% of those in LI, which is clearly a function of lower C content. Correlating in a further step soil OM with the EA reveals high associations between both elements shown below.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>α-glucosidase</strong></td>
<td></td>
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</tr>
<tr>
<td>00-10</td>
<td>92 Aa</td>
<td>114 Ab</td>
<td>148 Ac</td>
<td>97 Aa</td>
<td>92 Aa</td>
</tr>
<tr>
<td>10-20</td>
<td>64 Ba</td>
<td>73 Ba</td>
<td>77 Ba</td>
<td>68 Ba</td>
<td>71 Ba</td>
</tr>
<tr>
<td><strong>β-glucosidase</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>00-10</td>
<td>793 Aa</td>
<td>708 Aa</td>
<td>902 Aa</td>
<td>865 Aa</td>
<td>699 Aa</td>
</tr>
<tr>
<td>10-20</td>
<td>375 Ba</td>
<td>302 Ba</td>
<td>512 Ba</td>
<td>451 Ba</td>
<td>383 Ba</td>
</tr>
<tr>
<td><strong>neutral phosphatase</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>00-10</td>
<td>847 A b</td>
<td>3600 A a</td>
<td>1840 A a</td>
<td>1037 A b</td>
<td>877 A b</td>
</tr>
<tr>
<td>10-20</td>
<td>752 B b</td>
<td>957 B a</td>
<td>777 B b</td>
<td>760 B b</td>
<td>645 B c</td>
</tr>
<tr>
<td><strong>alkaline phosphatase</strong></td>
<td></td>
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<td></td>
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<tr>
<td>00-10</td>
<td>3647 A a</td>
<td>6137 A bc</td>
<td>6427 A b</td>
<td>5132 A c</td>
<td>3305 A a</td>
</tr>
<tr>
<td>10-20</td>
<td>3082 B a</td>
<td>4545 B b</td>
<td>3190 B a</td>
<td>3117 B a</td>
<td>2410 B ca</td>
</tr>
<tr>
<td><strong>protease (nmole tyrosin x g⁻¹ x h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>00-10</td>
<td>169 Aa</td>
<td>235 Ab</td>
<td>305 Ac</td>
<td>205 Ad</td>
<td>199 Ad</td>
</tr>
<tr>
<td>10-20</td>
<td>143 Ba</td>
<td>157 Bb</td>
<td>187 Bc</td>
<td>158 Bb</td>
<td>182 Bc</td>
</tr>
</tbody>
</table>

Values within a row followed by different lower case letters are significantly different (LSD p ≤ 0.05).

Values within a column followed by different upper case letters are significantly different (LSD ps 0.05).

<table>
<thead>
<tr>
<th>Correlations between soil OM and soil enzyme activities:</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{C - \beta\text{-glucosidase}} = 0.74^{**}$</td>
</tr>
<tr>
<td>$r_{C - \text{neutral}\text{-Phosphatase}} = 0.89^{**}$</td>
</tr>
</tbody>
</table>

Furthermore OM has noticeable relations with the soils’ “soluble” heavy metals:

$r_{C - Zn} = 0.84^{**}$ and $r_{C - Fe} = 0.87^{***}$
This fact is no wonder because composts have a high load of metals coming from diverse waste materials. Normally heavy metals have only a restricted concentration range where enzyme activities are positively influenced hence, it can be concluded that these relations between OM and enzyme activities are spurious correlations. Calculating the partial correlation coefficients ($r^2_p$) under exclusion of OM following results are achieved:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$r^2$</th>
<th>$r^2_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-glucosidase --&gt; Zn</td>
<td>+0.45*</td>
<td>-0.46**</td>
</tr>
<tr>
<td>β-glucosidase --&gt; Fe</td>
<td>+0.50**</td>
<td>-0.49**</td>
</tr>
<tr>
<td>Phosphatase (n) --&gt; Zn</td>
<td>+0.68***</td>
<td>-0.30*</td>
</tr>
<tr>
<td>Phosphatase (a) --&gt; Zn</td>
<td>+0.56*</td>
<td>-0.44*</td>
</tr>
<tr>
<td>Phosphatase (n) --&gt; P$_{soil}$</td>
<td>+0.30</td>
<td>-0.45*</td>
</tr>
<tr>
<td>Phosphatase (a) --&gt; P$_{soil}$</td>
<td>+0.30</td>
<td>-0.44*</td>
</tr>
</tbody>
</table>

Both examples demonstrate that on one hand side OM can act as "driving force" for enzyme activities in soils and on the other hand side can mask negative influences which are induced by heavy metals. The partial correlations reveal the negative effects of the heavy metals load of special compost material when incorporated in the soil. According to these findings two "in vitro" experiments were conducted with heavy metals.

1. *Effect of different metal ions on phosphatase activity*

   Soil samples from the control plot were incubated for phosphatase activity (s.a. Material and Methods). Further samples were incubated with salt solution (0.3 mM and 3.0 mM) of Zn, Mn, Cu, Mg, K, and Ni for 25 h. A second set was only incubated with aqua dest. for 24 h, and for 1 h with a salt solution, corresponding to the first set. The treatments are calculated relatively to the untreated set (Figure 1, left). Plant macronutrients (K, Mg) have no influence on enzyme activity neither in low nor in high concentrations. So called heavy metals Zn, Mn, Cu, and Ni result in different reactions. Zn, Mn and Ni show only nil to small reduction of activity at 0.3 mM. Except Cu which reduces activity for 25%, also 1h contact time reduces activity for 15%. At 30 mM K and Mg show no significant effects. With respect to Zn, Mn, Ni and Cu drastic declines of activities are observed. Most effective is Cu which reduces activity for 75% in the 25 h treatment; the 1 h incubation results also in a 50% decline.

2. *Effect of copper on neutral and alkaline phosphatase activity*

   The first experiment showed that Cu is most effective to reduce enzymatic activity in soils. Therefore, in series of Cu additions to phosphatase approaches from $1.1 \times 10^{-3}$ ppm up to $1.1 \times 10^4$ ppm Cu activities were measured for neutral and alkaline phosphatase. It can be seen that first activity reductions begin at 11 ppm Cu which aggravate at 110 ppm Cu (Figure 1, right). Thinking about soil health this range should never exceeded. In Germany a former proposal gave a threshold value of 40-60 ppm Cu (Bundesbodenschutzgesetz, 1998). These in vitro tests confirm more or less this range. Probably it is better to strive for lower values in order to save soil health. The copper input through agricultural activities is extensively described by Tamm et al. (2020).

![Figure 1](image-url). "In-vitro" experiments with differing heavy metals and plant nutrients on phosphatase activity. **Left.** Zn, Mn, Cu, Ni, K, and Mg. Concentrations: 0.3mM and 30mM. **Right.** Increasing Cu concentrations on activity of neutral and alkaline phosphatase.
CONCLUSION

Numerous studies exist about soil fertility including soil health and the importance of soil carbon (Engels et al. 2010, Oldfield et al. 2018). Well known textbooks describe all the possibilities for sound production and how to take care for the soil (Wild, A. 1988). Allison's monograph about soil organic matter gives a complex and complete overview about this important topic (Allison 1973). In the late sixties McLaren and Peterson (1967) began to publish a book series on soil biochemistry and ten years later Burns (1978) edited his book about soil enzymes. These ideas form a basis to see soils, soil fertility and quality in a new light (Garcia, C., Nannipieri, P., Hernandez, T. 2018). Especially the environmental services are most important.

Research in soil fertility has undergone a remarkable change by inclusion of soil health topics i.e. save the productivity of agriculturally used soils and look for external inputs which may jeopardize the complex system of fertility. In this context soil enzymes are good indicators of this well-balanced soil system. The results show very clearly that enzyme activities if active organic residues are incorporated in the soil or more or less "inert" organic matter. Seeing that in future huge amounts of agricultural waste material and other refuse will be treated by composting and brought back to the soils, control measures are needed to avoid a subtle intoxication of our soils.

In our "in vitro" experiment could be demonstrated that heavy metals in their ionic form are slow but severe acting toxins for soil biology. Insofar is it necessary that all materials which will get in the soil sphere, have to be controlled concerning their "soil toxicity". Interesting is the high capacity of soil organic material to mask the influence of those dangerous substances which could be clearly demonstrated with the help of spurious correlations.

That's beyond all questions soils will have an extraordinary relevance for the survival of our mankind: Firstly, guarantee that enough food will be available esp. for a growing mankind and secondly that a fully functional carbon sink will help to reduce the currently high atmospheric CO2 burden that mankind can further exist.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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