

# Ash additives to compost affect soil microbial communities and apple seedling growth

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## Aschezschlag zu Kompost beeinflusst die mikrobielle Gemeinschaft von Böden sowie Keimung und Wachstum von Apfelsämlingen

### 1 Introduction

Sustainability issues in agricultural policy are becoming of a high concern worldwide as it reflects the needs of long term fertility and environmental protection. Composting is a process of intensive treatment of organic wastes which involves optimization of biological degradation and stabilization of complex organic material. The major goal of composting is to provide a stable product that is high in nu-

trients which are easily plant available (GOBAT et al., 2003). Mature composts contain a diverse community of microorganisms, addition of compost to soil may considerably improve its biological, physical and chemical properties in short and long term and can confer plant disease suppressiveness (RYCKEBOER, 2003).

The annual increasing production of wood ash from biomass incineration plants that is disposed of in waste dumps is a source of concern. This ash could better be used as a sup-

### Zusammenfassung

In dieser empirischen Studie wurden die Auswirkungen von mit Asche versetzten organischen Abfällen als Substrat für die Kompostierung untersucht. Es wurde speziell die mikrobielle Gemeinschaft von Boden und Kompost untersucht, sowie das Potenzial einer Wachstumsstimulierung von Apfel-Jungpflanzen untersucht. Die Beimengung von Asche betrug 0 %, 8 % und 16 %. Der verwendete Kompost wurde einem Toxizitätstest unterzogen. Eine Zugabe von 8 % Asche zum Kompost hatte eine Veränderung der bakteriellen Gemeinschaft zur Folge und wirkte sich positiv auf die Verwertung von Kohlenstoffquellen in einem Substratnutzungstest (MicroResp™) aus. Bei 16 % Zugabe änderte sich zwar ebenfalls die bakterielle und pilzliche Gemeinschaft, eine Verbesserung der Kohlenstoffverwertung konnte jedoch nicht beobachtet werden. Keiner der Komposte verbesserte die Keimrate oder das Wachstum der Jungpflanzen signifikant. Die induzierten Veränderungen der Gemeinschaft und ihrer physiologischen Eigenschaften lassen darauf schließen, dass die Anwendung von Holzasche und organischen Abfällen in Apfelplantagen, bei abgestimmter Dosierung, durchaus möglich ist.

**Schlagwörter:** Kompost, Holzasche, Microresp, mikrobielle Gemeinschaft, Apfel-Sämlinge.

### Summary

In this empirical study, we studied the effect of ash admixture to organic wastes as a substrate for composting. In particular, we studied the microbial communities of composts and their potential to improve apple seedling growth. Amounts of 0 %, 8 % and 16 % ash admixture were used. All composts passed a toxicity test. Eight percent ash admixture to compost induced a shift in bacterial community and enhanced utilisation of all carbon sources (polymers, carboxylic and amino acids, alcohol, and carbohydrates) in a MicroResp™ assay, while 16 % ash admixture to compost changed bacterial and fungal communities but did not enhance C utilization. Neither of the composts significantly increased apple seed germination rate or seedling growth. The induced changes in community level physiological profiles and community composition suggest that with a more specific dosage of wood ash and organic wastes, use in apple orchard is possible.

**Key words:** Compost, wood ash, Microresp, microbial community, apple seedlings.

plement to fertilizers as it contains a variety of micronutrients and abundant potassium and calcium. Wood ash fertilization increases the pH and concentration of dissolved organic carbon (DOC) in the soil solution and enhances the activity of soil microorganisms (JOKINEN et al., 2006; MALJANEN et al., 2006). Wood ash has been shown to improve the composting process and may further enhance beneficial effects of composts (KUBA et al., 2008). An admixture of wood ash to the composting process might thus have particularly positive effects.

Apple replant disease (ARD) is a soil borne disease syndrome impairing tree growth and reducing yields when orchards are replanted (MAI et al., 1994). Old orchards are often low in organic matter, which may favour the buildup of soil pathogens in the replant site. Composts provide primarily two benefits to an orchard: First, the compost provides organic matter to the site that acts as a substrate enhancing beneficial microorganisms and that improves the soil humus status. Secondly, compost may serve as a carrier to add beneficial microorganisms to the soil (TRAVIS et al., 2003). Although the impact of ash amendment on soil microorganisms has been widely investigated by several authors (FRITZE et al., 2000; PERKIÖMÄKI and FRITZE, 2002), little has been done on microbial response to ash amendment on the community level.

The aim of this research was to investigate the influence of wood ash addition on compost microbial communities, and if composts with or without ash, might help to improve apple seedling germination and root elongation which might indicate a potential use in apple orchards and control of the ARD.

## 2 Material and methods

### 2.1 Study site and soil collection

Experiments were conducted with soil collected from a farm in Weiz (Austria, 47°04' N, 15°26' E). The orchard was originally planted with the apple variety *Gala* in 1983. The age of the second generation trees was 6 years at the time of sampling. Symptoms including plant shunting and leaf yellowing in an uneven pattern across a field were observed compared to other orchards. Sampling was conducted in December 2006. The soil was sampled in three randomly chosen areas, in the tree rows and the grass lanes, at a depth of 10 to 20 cm. Samples were bulked and sent to our laboratory. The soil was sieved (2 mm) and frozen at

-20 °C until use. Soil reserved for the greenhouse experiments was not sieved and not frozen.

### 2.2 Physical and chemical analysis

Background soil analyses were performed according to standard procedures. Total organic carbon ( $C_{org}$ ) was measured by dry combustion as described by INSAM (1996); and total N was determined by atomic absorption spectroscopy after wet acid digestion (ROS et al., 2006). Physico-chemical and biological characteristics are given in Table 1.

Table 1: Physico-chemical and biological properties of the test soil  
Tabelle 1: Physikalisch-chemische und biologische Eigenschaften des untersuchten Bodens

Humification rate (%)	3.3
pH	6.7
Sand (%)	30
Silt (%)	47
Clay (%)	23
P <sub>2</sub> O <sub>5</sub> (mg kg <sup>-1</sup> )	161
K <sub>2</sub> O (mg kg <sup>-1</sup> )	299
MgO (mg kg <sup>-1</sup> )	167
B (mg kg <sup>-1</sup> )	1.0
Organic carbon ( $C_{org}$ ) (g kg <sup>-1</sup> )	22.2 (0.45)
Total nitrogen (g kg <sup>-1</sup> )	2.31 (0.01)
C/N-ratio	9.61 (0.25)
Basal respiration [ $\mu$ g CO <sub>2</sub> -Cg <sup>-1</sup> soil h <sup>-1</sup> ]	8.08 (0.23)
Biomass [ $\mu$ g C g <sup>-1</sup> soil]	1758 (21)
Metabolic quotient [mg CO <sub>2</sub> -Cg <sup>-1</sup> soil h <sup>-1</sup> ]	1.255

Numbers in parentheses indicate the standard deviation, n = 5.

### 2.3 Soil microbial basal respiration and biomass carbon

Basal respiration was measured as CO<sub>2</sub> evolution from moist soil samples (60 % WHC) at 22 °C using a continuous flow infrared system (HEINEMEYER et al., 1989). Readings were taken after 14 h incubation. Microbial biomass carbon was determined by substrate induced respiration (SIR), as described by SPARLING (1995) after addition of 1 % glucose (dry matter basis) to the samples and measuring CO<sub>2</sub> evolution for 6 h.

### 2.4 Compost origin and characteristics

The different composts were supplied by the composting plant Weer (Tyrol, Austria). The substrates were communal

biowaste and tree-bush-cutting (53:47) (W/W), with 0 %, 8 %, and 16 % wood ash, labelled K0, K8 and K16. The ash was obtained from the combined heat and power plant Kufstein (Austria) that works with bark, sawdust and wood chips as input materials. Ashes were added at the beginning of the composting process. The composts fulfilled the requirements for general use in agriculture according to the Austrian compost ordinance and were previously used for research purposes (KUBA et al., 2008). The heavy metals contents are listed in Table 2.

Table 2: Heavy metal contents of composts prepared without (K0) and 8 % (K8) and 16 % (K16) ash admixture (mean  $\pm$  SD)

Tabelle 2: Schwermetallgehalte der Komposte, ohne (K0), mit 8 % (K8) und mit 16 % (K16) Asche-Beimengung (Mittelwert  $\pm$  SD)

	K0	K8	K16
Lead	58.0 ( $\pm$ 2.9)	39.3 ( $\pm$ 2.4)	34.6 ( $\pm$ 4.2)
Cadmium	0.8 ( $\pm$ 0.1)	0.6 ( $\pm$ 0.1)	0.7 ( $\pm$ 0.1)
Chromium	25.2 ( $\pm$ 13.7)	27.1 ( $\pm$ 2.8)	27.9 ( $\pm$ 0.2)
Copper	67.3 ( $\pm$ 6.7)	66.6 ( $\pm$ 11.3)	55.4 ( $\pm$ 1.9)
Nickel	16.4 ( $\pm$ 1.4)	22.1 ( $\pm$ 10.7)	20.6 ( $\pm$ 2.0)
Mercury	0.4 ( $\pm$ 0.1)	0.2 ( $\pm$ 0.1)	0.2 ( $\pm$ 0.1)
Zinc	181 ( $\pm$ 19)	183 ( $\pm$ 24)	189 ( $\pm$ 7)

Numbers in parentheses indicate the standard deviation,  $n = 3$ .

## 2.5 Community level physiological profiles of composts

The community level physiological profiles (CLPPs) were determined by using a micro-respiration technique (Microresp<sup>TM</sup>; by CAMPBELL et al., 2003) with 22 carbon sources (RUBATSCHER et al., 2006) (see Figure 2). The microplates were read before and after 6 h incubation at a wavelength of 570 nm with a Zenyth 3100 (Anthos, Wals, Austria) spectrophotometer. The data were analysed by principal component analysis (PCA); principal components were subjected to one-way ANOVA and Tukey B test at  $P \leq 0.05$  level of significance (GOBERNA et al., 2005). The statistical analyses were performed using SPSS15 (SPSS<sup>®</sup>, Chicago, USA).

## 2.6 Toxicity evaluation of composts

Toxicity testing of compost extracts towards germination of apple seed and root growth was done according to LAU et al. (2001). Extracts were prepared by shaking composts with deionised water at a 1:2 (w/v) ratio on an orbital shaker at 180 rpm for 1 h, followed by centrifugation at

3,000 rpm for 20 min. The extract was then filtered through 0.45 mm Millipore membrane filters, and was then used undiluted or diluted with deionised water to 5 % or 50 % concentration. Ten seeds were placed on a double layer of filter paper, placed in 90 mm sterile plastic Petri dishes containing 4 ml of the compost extracts at 5, 50, or 100 % of the original concentration. The Petri dishes were covered with tinfoil and incubated at 25 °C. Deionised water was used as the control treatment. To keep the moisture constant, three Erlenmeyer flasks containing 100 ml of deionised water were placed in the incubator. All treatments were performed in triplicate. After 6 days, the number germinated seeds were counted and the lengths of roots were measured.

The percent growth inhibition was calculated as follows:  

$$\text{Inhibition (\%)} = (L_{\text{control}} - L_{\text{treatment}}) / L_{\text{control}} * 100$$
 where  $L_{\text{control}}$  is the root length in the control, and  $L_{\text{treatment}}$  the root length treated with compost extracts.

## 2.7 Growth test with apple seedlings

Growth tests with apple seedlings (cv. Granny Smith) were performed according to MANICI et al. (2003) (modified). Apple seeds were stratified at +4 °C for 31 d, and then they were sown in a sterile peat and sand mixture (3:1) and maintained at 18–22 °C for 21 d. Seedlings were transferred to holes (7 cm deep, 7cm diameter) filled with a mixture of compost and soil from the apple orchard 1:3 (W/W). The trial was arranged in a completely randomised block design with four replicates. Treatments applied were: K0, K8, and K16. A set of control plots was left untreated.

Seedlings were grown at 26–32 °C in a greenhouse for 40 d and then harvested. The growth score (GS) was assessed by multiplying plant height (above ground part) by plant health score (0: dead plants, 1: very weak, 2: weak, 3: healthy) (MANICI et al., 2003). Transformed data [ $\log(x+1)$ ] were subjected to univariate analysis of variance (ANOVA), mean values among treatments were compared by the Tukey B test at  $P \leq 0.05$  level of significance.

## 2.8 Extraction of DNA and PCR

DNA extraction was performed for the different subsamples of composts and soils using the Fast DNA Spin Kit for soil (BIO 101, USA). Isolation was performed on 0.25 g samples following the manufacturer's instructions. PCR

amplification targeting the 16S rDNA for Bacteria and 18S rDNA for fungi was performed in a thermocycler (Flexcycler, AnalytikJena, Germany), using the universal bacterial primers F984 (3'AACGCGAAGAACCTTAC 5') (EICHNER et al., 1999) and R1378 (3'CGGTGTGTACAAGGCCGGGAACG 5') (HEUER et al., 1999); and the fungal primers FF390 (3' CGATAACGAACGAGACCT 5') and FR-1GC (3'AIC CAT TCA ATC GGT AIT5') (VAINIO and HANTULA, 2000). A GC-clamp was added to stabilize the melting behavior of DNA (MUYZER et al., 1995). Products of approximately 433 bp and 389 bp were obtained with the bacterial and fungal primers, including a 40 bp GC-clamp. Each PCR mixture contained 1 µl of extracted DNA, 2.5 µl of buffer (15mM MgCl<sub>2</sub>), 0.5 µl of MgCl<sub>2</sub> (50 mM), 0.5 µl of each primer, 0.5 µl of dNTPs, 1 µl of DMSO, 0.5 µl of BSA, 0.25 µl of Taq polymerase and 19.25 µl of purified water to a total volume of 25 µl.

The PCR program for bacteria included an initial 3 min denaturation step at 94 °C and was followed by 26 cycles of 1 min at 94 °C, 1 min at 62 °C and 2 min at 72 °C. The thermal cycling was completed with an extension step at 72 °C for 4 min.

Fungal DNA was amplified using 5 min denaturation at 94 °C, followed by 35 cycles of 30s at 94 °C, 30s at 56 °C and 1 min at 72 °C. The thermal cycling was completed with an extension step at 72 °C for 10 min. PCR products were examined by agarose gel electrophoresis (1.5 % agarose; 0.50 TAE gel [50 TAE is 2M Tris, 0.05M EDTA-Na<sub>2</sub>, 1M Acetic acid glacial, pH 8.5]) with standard ethidium bromide staining to check for recovery of products of the expected size.

## 2.9 PCR product quantification and DGGE analysis

PCR products were quantified as described by JÜEN and TRAUOGOTT (2005). DGGE was done according to DUINVELD et al. (2001) with a denaturing gradient of 30 to 60 % of the denaturant, using 7.5 % acrylamide gels. A product equivalent to 60 ng of PCR products were loaded in triplicate, and DGGE was performed in 1 Tris-Acetate-EDTA buffer at 60 °C using an electrophoresis tank (INGENYphorU, Ingeny International, Goes, Netherlands) at a constant voltage of 100 V for 16 h (Unipack 2000 Power supply, UniEquip, Munich, Germany).

The gels were then silver stained according to HEUER et al. (1999) (Hofer Automated Gel stainer, Amersham Pharmacia Biotech, Uppsala, Sweden), air dried and scanned

with the Epson perfection scanner (Epson, Klosterneuburg, Austria). The GelCompar II program (Applied Maths, Ghent, Belgium) was used to analyze the bacterial and fungal community fingerprints of each denaturing gradient gel as described by SMALLA et al. (2001).

## 3 Results

### 3.1 Physico-chemical and biological parameters of soil and composts

Physico-chemical data of the soil, as well as basal respiration, microbial biomass and the metabolic quotient are given in Table 1. Heavy metal concentrations of the three composts are given in Table 2.

### 3.2 Toxicity evaluation of composts

The germination percentage and percent growth inhibition index of apple seeds were determined in compost extracts at different concentrations. At 5 % concentration, all compost extracts showed a 100 % germination rate (Figure 1). The rate decreased below 100 % at 50 % extract concentration for K8 and K16, and at 100 % concentration the germination rate decreased for all composts. Compared to the control, the undiluted compost extracts as well as the 50 % extract inhibited root elongation up to 25 %. The strongest inhibition was observed for the compost without ash and the one that had received 16 % ash. The dilute compost extract of the ash amended composts, and in particular the one with 8 % ash, stimulated root elongation by 16 %.

### 3.3 Community level physiological profiles of composts

For all types of substrates microbial communities in K8 showed higher respiration rates than those in K0 and K16 (Figure 2). ANOVA showed significant differences among the three composts regarding the two principal components. PC1 explained 89.4 % of the variance and was most closely correlated to the activities induced by polymers, carboxylic and amino acids, alcohol, and carbohydrates (in decreasing order). Along PC2 (correlating with N-acetyl D-glucosamine and explaining 5.8 % of the variance), K8 and K16 were similar, but were differed to K0 (Figure 3).

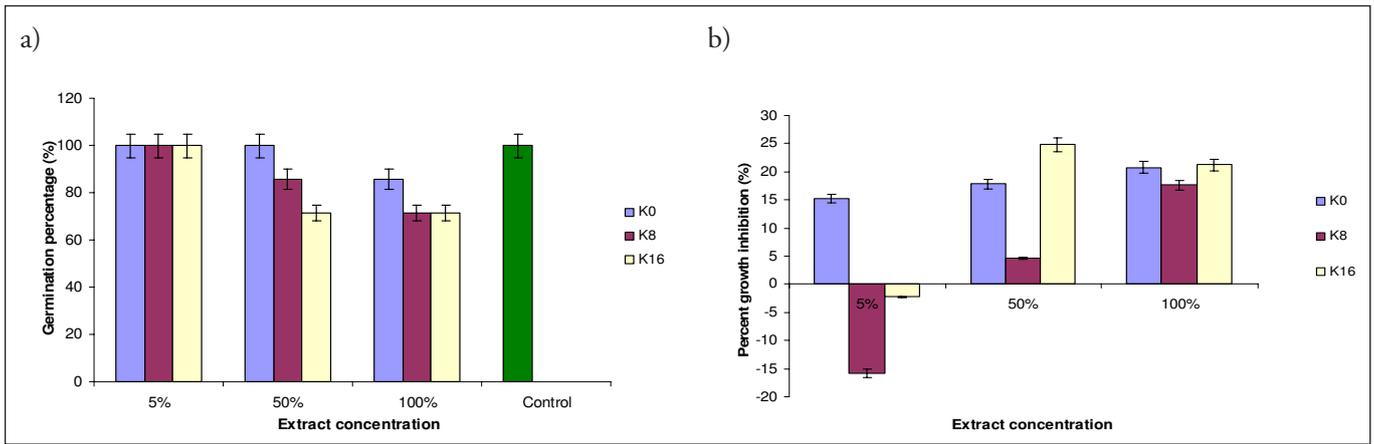


Figure 1: The germination percentage and percent growth inhibition index of apple seeds in compost extracts at different concentration. The error bars indicate the least significant difference at  $P < 0.05$

Abbildung 1: Die prozentuelle Keimungsrate und der Wachstums-Inhibierungs-Index von Apfelsämlingen bei Behandlung mit Kompostextrakten verschiedener Konzentrationen. Die Fehler-Balken zeigen die geringste signifikante Differenz ( $P < 0.05$ )

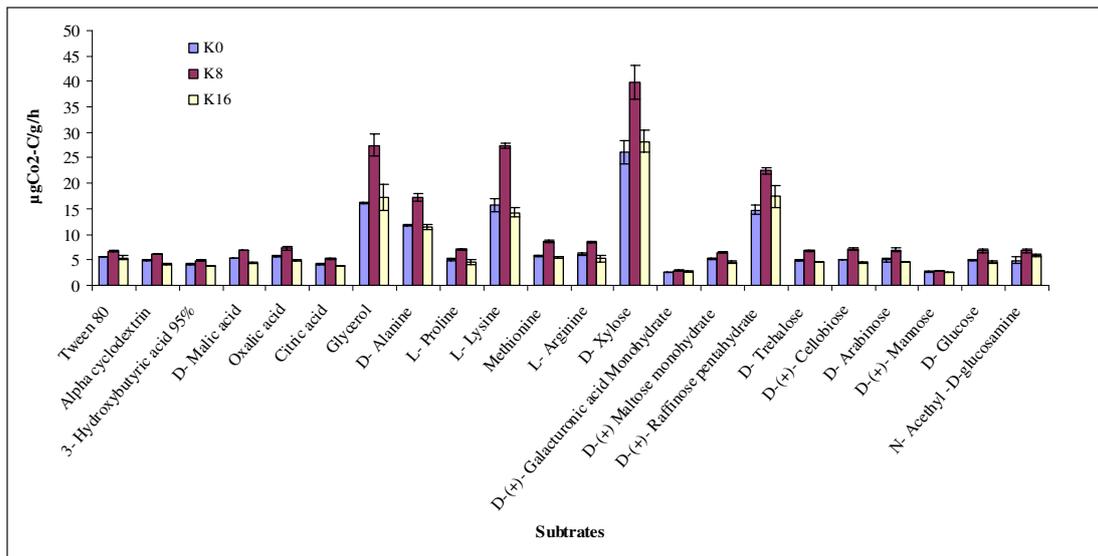


Figure 2: Substrate-induced respiratory responses for 22 carbon sources in composts prepared without (K0) and 8 % (K8) and 16 % (K16) ash admixture, as determined by the MicroResp method. The error bars indicate standard deviation ( $n = 3$ )

Abbildung 2: Substratinduzierte Atmung (MicroResp-Methode) mit 22 Kohlenstoffquellen in Kompost ohne (K0), oder mit 8 % (K8) und 16 % (K16) Asche-Beimengung. Die Fehler-Balken zeigen die Standardabweichung ( $n = 3$ )

### 3.4 Microbial community composition

For the composts, fungal and bacterial community profiles of K16 differed from those of K0 and K8 (Figure 4) which showed more than 99 and 95 % similarity, respectively. Fungal and bacterial communities from experimental soils showed a clear influence of composts. For both bacterial and fungal communities, additional bands were found due

to compost amendment, but some were lost. Some of the additional and lost bands found after compost amendment are highlighted in Figures 5a and 5b. Fungi seemed to be more sensitive to the increasing amount of ash admixture (similarity higher to K8 than to K16) in both composts and green-house experiments (Figure 6).

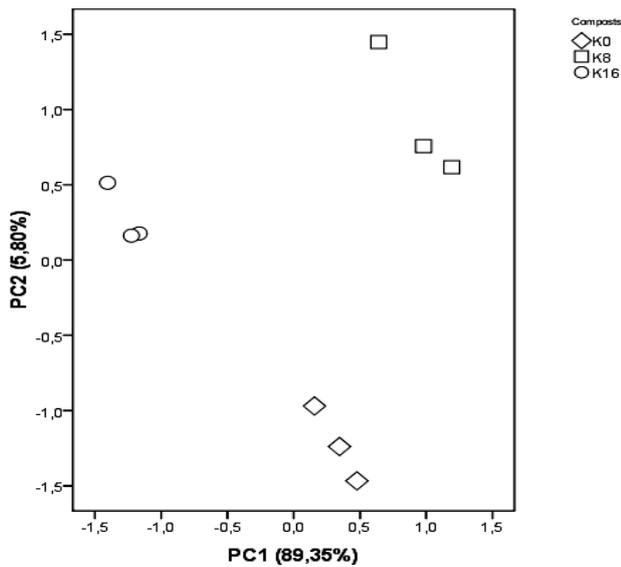


Figure 3: Principal component analysis plot showing distinct differences in the community level physiological profiles (Microresp method) among the composts produced without (K0), 8 % (K8) and 16 % (K16) ash

Abbildung 3: Hauptkomponentenanalyse: die Graphik zeigt deutliche Unterschiede im physiologischen Profil (Microresp Methode) zwischen den Komposten ohne (K0) und mit 8 % (K8) oder 16 % (K16) Holzasche

### 3.5 Growth test with apple seedlings

The growth score of apple seedlings (after univariate analysis of transformed data [ $\log(x+1)$ ],  $P \leq 0.05$ ) did not show a significant difference among the treatments. However, a trend for a higher growth score for K0 and K8 treatments was observed, while K16 showed a trend towards a decreased growth score compared to the control.

## 4 Discussion

### 4.1 Physico-chemical and biological parameters of soil and composts

There was no indication that the lack of a particular micro- or macronutrient, pH, or humification rate could explain the problems with replanting. The C: N ratio of the soil (9.61) was within the range normally found. Basal respiration as well as microbial biomass were higher than usually found in many soils, while the metabolic quotient ( $1.25 \text{ mg CO}_2\text{-Cg}^{-1} \text{ soil h}^{-1}$ ) was low, indicating a balanced soil microbiota unlikely to be subject to any stress (INSAM et al., 1996).

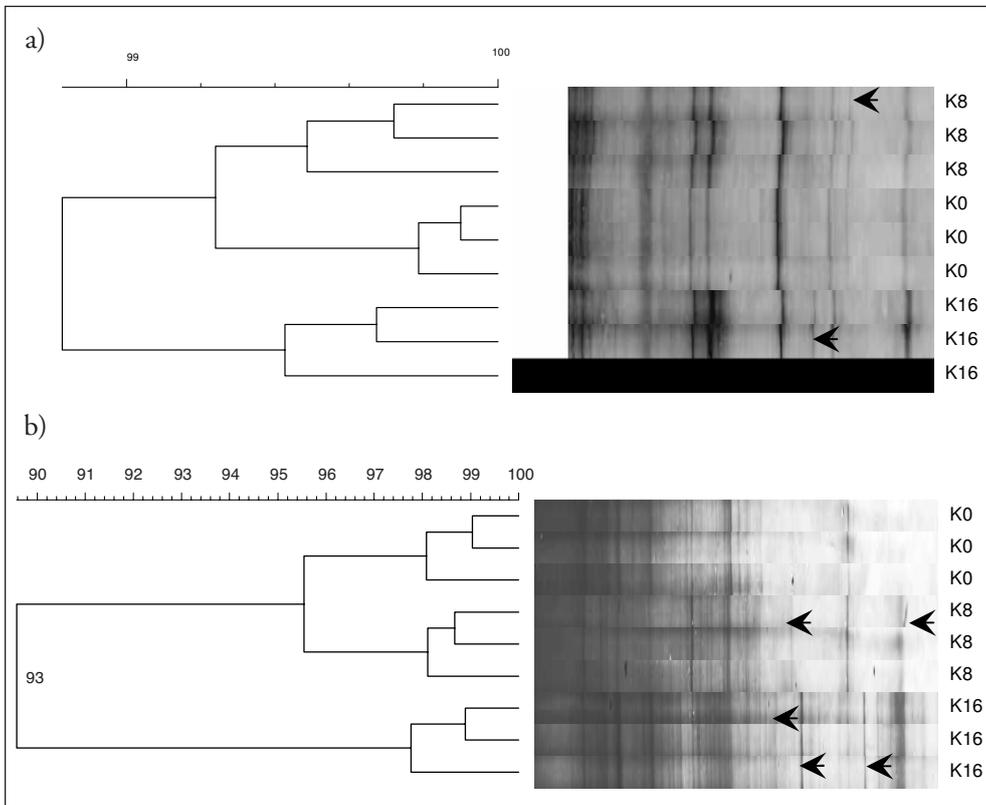


Figure 4: Hierarchical cluster analysis of DGGE gel images of the fungal (a) and bacterial (b) communities of different composts. The scale in the dendrogram indicates percent similarities

Abbildung 4: Hierarchische Cluster-Analyse eines DGGE-Gels von verschiedenen Komposten mit (a) bakteriellen und (b) pilzlichen Gemeinschaften. Die Skala im Dendrogramm gibt die prozentuelle Similarität an

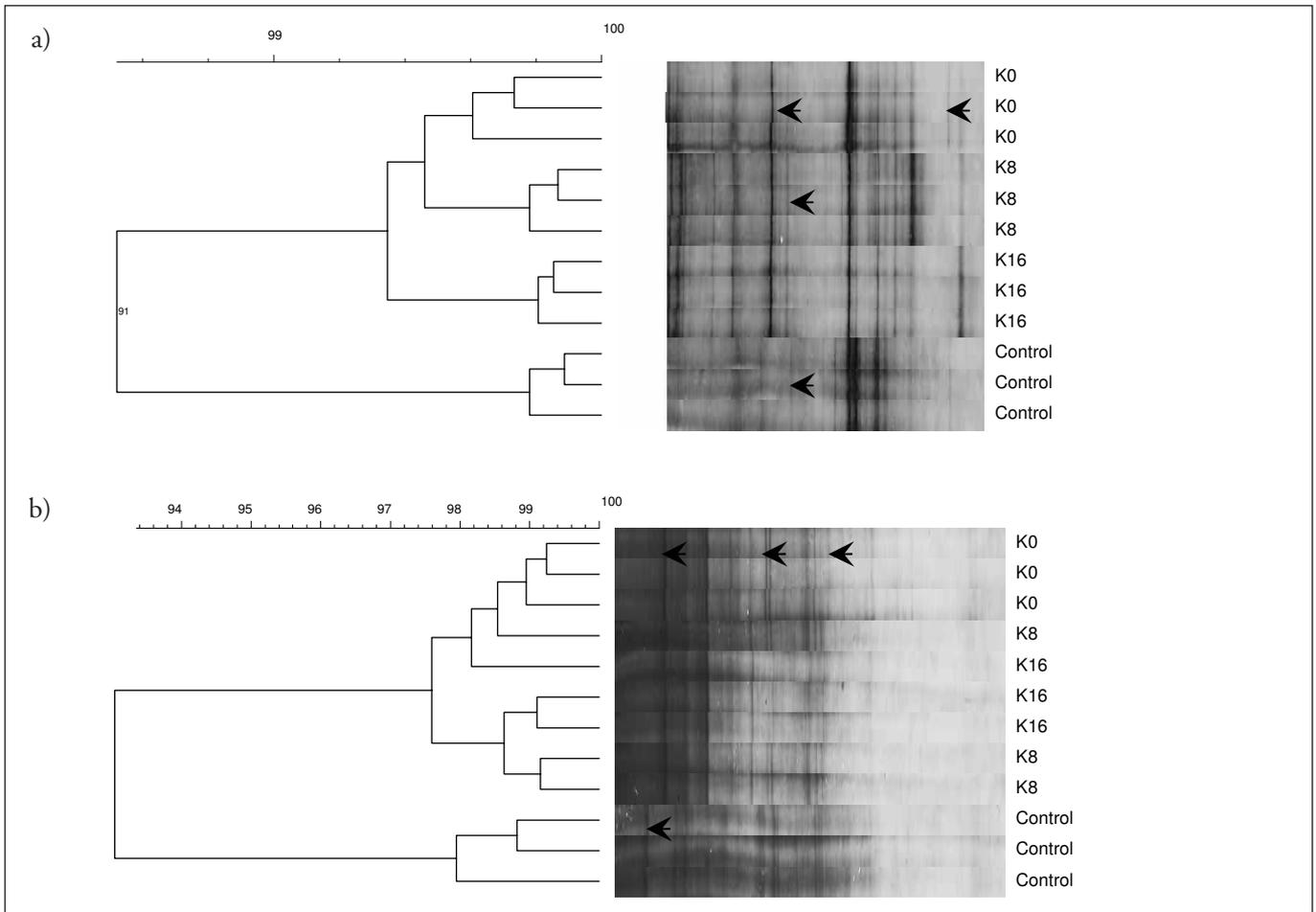


Figure 5: Hierarchical cluster analysis of DGGE gel images of the fungal (a) and bacterial (b) communities among treated soils after harvesting. The scale in the dendrogram indicates percent similarities

Abbildung 5: Hierarchische Cluster-Analyse eines DGGE-Gels zwischen behandelten Böden nach der Ernte der (a) bakteriellen und (b) pilzlichen Gemeinschaften. Die Skala im Dendrogramm gibt die prozentuelle Similarität an

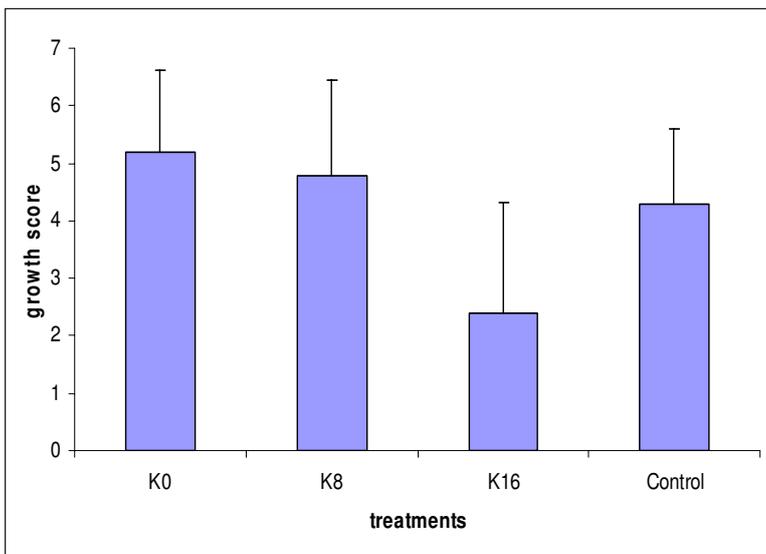


Figure 6: Growth score of apple seedlings in a soil treated with composts prepared without (K0) and 8 % (K8) and 16 % ash admixture. Vertical bars indicate standard deviation (n = 12)

Abbildung 6: Wachstumsrate von Apfel-Jungpflanzen in einem mit Kompost ohne (K0), mit 8 % (K8) und mit 16 % (K16) Asche behandelten Boden. Die vertikalen Balken geben die Standardabweichung an (n = 12)

## 4.2 Phytotoxicity test

The use of a phytotoxicity test is essential to evaluate the acceptability of organic waste compost for agricultural use (LAU et al., 2001). The stimulation effect observed at 5 % extract concentration might be due to the low EC resulting in less potential inhibitory effect of ash (WALSH et al., 1991, INSAM et al., 2009). Also LAU et al. (2001) state that in waste composts an addition of 5 to 20 % ash could reduce the potential toxicity from available metals. It has further been reported that an application of small amounts of ash (5–10 %) to soils may enhance seed germination as well as seedling growth (SINGH et al., 1997). Even with the undiluted extracts, the maximum inhibition of root growth did not exceed 25 %. It is not known, however, if the roots are the most sensitive part of the soil-plant system in this case. For example, in a study by HAIMI et al. (2000) effects of wood ash on the soil microflora were evident. Several authors investigated the effects of wood ash on the soil fauna. For example, wood ash effect on the number of collembola was neutral (HAIMI et al., 2000), positive (HUHTA et al., 1986) or negative (LIIRI et al., 2002).

Our data indicate that compost with a moderate level of wood ash (8 %) could have clearly positive effects on plant seedling germination and root elongation, supporting findings of KUBA et al. (2008) while 16 % ash might be beyond the optimum.

## 4.3 DGGE

Wood ash admixture did not affect the dominant groups of fungi in compost (more than 98 % similarity with both composts), but the dominant bacterial communities were affected, in particular in K16 (less than 90 % similarity). However, it is difficult to judge what happened with the minor groups as DGGE detects only dominant species (MUYZER et al., 1995; DUINEVELD et al., 2001).

All the compost treated soils differed to the control and both fungal and bacterial groups were affected, confirming earlier observations by ROS et al. (2006) and INNEREBNER et al. (2006) who found that composts change soil microbial community composition. The difference in the effects of ash- versus non-ash composts is in accordance with other authors (FRITZE et al., 2000; PERKIÖMÄKI and FRITZE, 2002). The observed perturbations may be attributed to dissolved oxides such as MgO, CaO and BaO released from ash which increase the pH and may deactivate

microorganisms (BAATH et al., 1995; PAPADIMITRIOU et al., 2006).

## 4.4 CLPPs

Compost and ash application is known to enhance microbial activity and biomass (INSAM and MERSCHAK, 1997; BORKEN et al., 2002). In a field experiment the strongest stimulation of microbial biomass and activity was observed after application of compost with 8 % ash (KUBA et al., 2008). Soil microbial activity and biomass enhancement after ash application may be attributed to pH changes and an increased concentration of dissolved organic carbon (DOC) (JOKINEN et al., 2006). Increased abundance of many bacterial groups was observed by KARSISTO (1979) and WEBER et al. (1985) after ash application.

Substrate-induced respiration in the CLPP assay was enhanced in the composts with 8 % ash supporting the earlier observation that this amount of ash may have a particular positive effect on microbial activity, while an amount of 16 % may have adverse effects (KUBA et al., 2008). The changes in the CLPPs due to ash amendment further indicate potential effects on the functional properties of composts.

For composts, little is known about the relationship between humus quality and the involved microbiota. The interrelationship between microorganisms and humus quality in soils has, among others, been investigated by DINEL et al. (1998) and more recently by JOKINEN et al. (2006) and KLAMMER et al. (2008). In a study investigating more than 100 composts, Jacques Fuchs (personal communication) found that composts that had undergone an anaerobic phase were more likely to confer plant disease suppression than strongly aerated composts. It is still not known if it is the microflora indigenous to the composts that confers plant disease suppressiveness or if it is the triggering of a specific soil-based community by the compost that confers such effects. There are numerous examples of composts that are able to protect different types of plants against certain parasites (FUCHS and LARBI, 2004). The choice of appropriate compost for a certain purpose is of high importance. Wrong compost application (e.g. an excess of compost) can result in a negative effect. It has also been observed that composts that have been cured for too long a time may lose their disease suppressiveness (DANON et al., 2008).

#### 4.5 Plant seedling growth score

Neither the direct effects on the soil chemistry, nor indirect effects through an altered soil microbial community changed the germination and growth of apple seedlings. CHEN et al. (1988) suggested that increasing microbial activity by compost amendment may infer disease suppression. The trend of a decrease in the growth score with K16 may not be substantiated with the available dataset. The observation that ash may have a dual effect by promoting and inhibiting growth in a dose-dependent manner (GUPTA et al., 2002) suggests that more different compost compositions should be tested. The inhibitory effect of ash could be attributed to the release of metal elements from mixture components to the soil and their consequent uptake by the plants (FUENTES et al., 2004). This is supported by the phytotoxicity test results where a decrease of seed germination was linked to an increase of ash concentration. Several authors (e.g., ETIEGNI and CAMPBELL, 1991; SOMESHWAR, 1996) stated that beneficial or adverse effects of wood ash depend on characteristics of input materials (type of plant, part of plant combusted, type of waste, condition of combustion, collection and storage), for that reason finding a balance between nutrient deficiency in soil and toxic and beneficial compounds in ash for suitable growing conditions in agriculture is needed.

#### 5 Conclusion

The aim of this study was to investigate the microbial community and apple seedlings response to the ash and amendment of composts that had been produced with or without addition of ash. Our results showed that ash admixture to compost changed the microbial community composition and increased the microbial CLPPs. Some positive trends on seed germination, root growth, and plant growth were found for the composts without and with 8 % wood ash admixture, while 16 % ash admixture was beyond the optimum.

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