Use of wood ash and anaerobic sludge for grassland fertilization: Effects on plants and microbes

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Wirkung von Holzasche und Gärrest auf Pflanzen und Mikroorganismen bei der Melioration von Grünland

1 Introduction

Increasing the share of renewable energies is among the prime policies of the European Union. Among the options are the incineration of wood in combined heat and power plants as well as the anaerobic digestion of organic materials to obtain biogas. Both processes leave behind residual matter – wood ash and anaerobic sludge. These residual materials contain chemical elements with considerable fertilizer value, but can also contain heavy metals, xenobiotics and may emit odorous components. Wood ash is often deposited in waste dumps at considerable expense, and the increasing centralization of biogas plants results in problems for the distribution of the residual sludge, with not enough agricultural land being in the vicinity of the biogas plants.

Wood ash has traditionally been used as fertilizer. Some studies have shown that grate (bottom) ashes may be used as fertilizer in agriculture and forestry (FRITZE et al., 2000). The ability of ashes to increase soil pH by oxides, hydroxides and carbonates of K, Mg and Ca is an advantage for the treatment of acidic soils (HOLZNER and OBERNBERGER, 1998; HOLZNER, 1999). DEMEYER et al. (2001) found a positive effect of ashes on soil texture, aeration, water holding capacity and cation exchange capacity. The gener-

Zusammenfassung

Das Ziel der vorliegenden Studie war die Überprüfung der Wirkungen von Aschen, Gärresten und Mischungen davon auf die Mikrobiologie des Bodens und das Pflanzenwachstum sowie auf die Nitratauswaschung. Weiters sollte untersucht werden, ob mögliche Probleme durch erhöhten Salzgehalt oder erhöhte Alkalinität durch die Zugabe des Tonminerals Vermiculit vermindert werden können. Der Effekt von Gärresten und Aschen auf die klassischen mikrobiellen Parameter war gering, es zeigte sich jedoch eine signifikante Änderung der Zusammensetzung der Mikroflora des Bodens. Bei Zusatz von Gärrest entsprechend einer Düngermenge von 180 kg N ha^{-1} konnte eine hohe Nitratauswaschungsrate festgestellt werden. Diese wurde durch die Zugabe von Asche und Vermiculit nicht verringert. Durch die Zugabe von Gärrest kam es zu einer Keimverzögerung bei Kresse, dieser Effekt konnte durch Zugabe von Vermiculit verringert werden.

Schlagworte: Gärrest, Holzasche, Nitratauswaschung, Dünger, Biogas.

Summary

The aim of this study was to investigate if the use of wood ash and sludge, or a combination of them, could change soil microbiological properties and plant growth. A second aim was to determine if high doses of these amendments could create problems in terms of nitrate leaching, and if such problems could be offset by adding a clay mineral, vermiculite. The effect of sludges and ash on soil microbial bulk parameters was found to be small, while the composition of the bacterial community was considerably changed. We were able to demonstrate that an application rate of sludge corresponding to 180 kg N ha^{-1} was problematic in terms of nitrate leaching and could not be offset by amending with ash or vermiculite. Germination of cress was retarded by sludge and ash addition, however, this effect was partly offset by the addition of vermiculite.

Key words: Anaerobic sludge, wood ash, organic fertilizer, lysimeter, nitrate leaching.

al notion is that fertilization with ashes has short term effects lasting for approximately one year (FRITZE et al. 2000; ZIMMERMAN and FREY, 2002). Phosphate solubility in ashes is low (VESTERINEN 2003) and according to MOILANEN et al. (2006), application of ash promotes plant growth only if there is no N limitation. If ashes are considered to be used as fertilizers, their heavy metal content needs to be limited (NEURAUTER et al., 2004). However, no EU heavy metal limit values exist (VESTERINEN 2003). In Austria, the limits according to the compost ordinance are decisive (Table 1).

According to several authors (ARONSSON and EKELUND, 2004; DEMEYER et al., 2001; FRITZE et al., 2000; PERKIÖMÄKI and FRITZE, 2002; ZIMMERMANN and FREY, 2002), ash amendment increases soil microbial activity and biomass. In contrast, however, ash amendment to soils has also been found to inhibit fungal growth (BAATH et al., 1995). PERUCCI et al. (2006) studied the effect of addition of wood ash at 5 and 20 t ha^{-1} on soil microbial and biochemical properties. The soil microbiological biomass (C and N), and general microbial activity increased at the lower wood-ash dose, whereas microbial biomass C decreased at the higher ash dose. A changed microbial C/N ratio of treated samples suggested changes in the structure of the microbial communities. The nutrient content of anaerobic sludge depends, to a large degree, on the original contents of the substrates from which the sludge was produced (PFUNDTNER, 2005). Due to C losses during anaerobic digestion through the formation of CH_4 and CO_2, the original C/N ratio changes from 9 to 6 (AMON and DÖHLER, 2005). Up to 88 % of the N is present as ammonium (PÖTSCH, 2005). At the usual hydraulic retention times employed, anaerobic degradation fails to properly digest the poorly degradable fractions such as cellulose and lignin, as well as xenobiotics. Thus, their concentration, and that of macro- and micronutrients (also heavy metals), is higher than in undigested manure. In an anaerobic sludge, 5–7 % of N, 30–50 % of P and 70–100 % of K, respectively, are available to plants in the first year (KIRSCH, 2002). Nitrogen availability from digested liquid manure is much higher from of undigested manure (PFUNDTNER, 2005) and the fertilizer value of digestates is regarded to be superior to that of undigested manure (AMON and DÖHLER, 2005).

The degradation of organic acids results in the pH increasing by up to 0.5 units, so that cattle and pig manure digestates show a pH of approximately 8 (PÖTSCH, 2005). Heavy metal contents in biowaste are usually low (PFUNDTNER, 2005), however, in 15 % of the anaerobic sludges analysed in an Austrian study, it was found that the Cd contents exceeded the current limit (Austrian Compost Ordinance) of 3 mg Cd kg^{-1} dm^3 (PÖTSCH, 2005) (Table 1). It is also known that Cu and Zn from pig manures can cause soil hazards (MORAL et al. 2008). However, anaerobic treatment does not result in an increase of the total load of these elements upon application to soils.

Small scale biogas plants and wood incineration plants can often be found in operation in proximity to each other. Various benefits exist from the combination of the two waste types. Firstly, anaerobic sludge may be used for carbonisation of the wood ash. The increase in pH may however result in loss of gaseous nitrogen (HOLZNER and OBERNBERGER, 1998). This could be avoided by a rapid incorporation into the soil. In a lysimeter experiment with corn, slightly higher ammonia emissions were found following the combined application of ash and anaerobic sludge to soil, and plant growth was improved compared to the application of sludge or mineral fertilizer alone (WENZEL and ZIVKOVIC, 2006). The admixture of coal ash to sewage sludge has been commonly used to improve soil nutrient balance (DIMITROU et al., 2006). In Sweden, it is common to use wood ash in combination with sewage sludges for the fertilization of energy forests (DIMITROU et al., 2006), resulting in an increase of soil pH, and a reduction of Cd-, Ni- and Zn-concentrations in the soil. When sewage sludge was used without the ash, the heavy metal uptake of plants increased compared to the control.

Composting of organic materials with admixture of wood ash has proven to be an ecologically sound and agronomically acceptable way of recycling ash (KUBA et al., 2008). Given that organic wastes and anaerobic sludge have a similar elemental composition, it is possible that the application of anaerobic sludges and wood ash to soils may also be a good method of recycling and disposing of these nutrient-rich by-products. The aim of this study was to investigate the combined use of wood ash and anaerobic sludge from biogas plants as a fertilizer. In a lysimeter experiment, we studied the effects on the soil microbiota, nutrient leaching and effects on plant growth. We further investigated the ability of vermiculite to reduce potential negative effects by high salt loads of ash or sludge. Vermiculite is known to increase the active surfaces in soils and the retention of ions (DOEGE, 2002).

2 Material and methods

2.1 Ash, anaerobic sludge, seeds, and soil

We used bottom ash from the combined heat and power plant in Kufstein (Austria), where bark, saw dust and wood chips in equivalent amounts are incinerated. The remaining ash was carbonized by admixing water. The C content of the ash was 4.4 %, the pH was approximately 12.3, and the electrical conductivity ranged from 5.5 to 7.8 mS cm^{-1}. Heavy metal contents are given in Table 1. The vermiculite used in the study was obtained from Dehner, Völs, Austria. The seed mixture "SR057: Böschungsrasen ALPIN (B3)" (Schwarzenberger, Völs, Austria) was used and applied at a rate of 350 kg ha^{-1}, corresponding to 0.33 g per lysimeter, containing 85 % grass seeds, and 15 % legume seeds (details in KUBA, et al. 2008, 43–49).

Anaerobic sludge was obtained from Biowatt–Taufers GenmbH (Kematen, Italy). The plant was run at 50 °C and was comprised of a main fermenter and a post-fermenter (1000 m^3 volume each). Input material was cattle manure (85 m^3 d^{-1}), apple residues (25 t d^{-1}) and finely ground rice hulls (added in small amounts). Sludge was sampled on July 24, 2006 from the storage tank following digestion, and stored at –18 °C until use. The dry matter content of the sludge was 8.43 %. The C and N contents of the dry matter were 35.2 % and 2.3 %, respectively. The pH was 7.98, and the electrical conductivity was 23.9 mS cm^{-1}. The soil for the mini-lysimeters was taken on September 29, 2006, from an alpine meadow Eutric Cambisol at 1960 m above sea level (Kaserstattalm, Central Alps, Tyrol, Austria) (BITTERLICH and CERNUSCA, 1999).

2.2 Experimental setup

Plexiglass tubes (mini-lysimeters; 30 cm high, 11 cm diameter) were filled to 24 cm with soil, and the remaining 6 cm were filled with the soil/substrate mixture. Substrate materials were mixtures of anaerobic sludge, ash and vermiculite at an N – equivalent rate of 180 kg N ha^{-1}. Sludge was used at an amount of 91.2 g per lysimeter (corresponding to a rate of 96 t ha^{-1}). Ash was used in two different amounts, (1) similar to the sludge dry weight, 7.75 g per lysimeter – SA1, and (2) a triple amount, 23.3 g per lysimeter – SA3. To offset potential nutrient overload, vermiculite was added at an amount of 23.3 g per lysimeter (Table 2).

The columns were watered weekly with 100–200 ml deionised water and kept at 22 °C. The percolate was collected and used for ammonium and nitrate analysis. After 8 wk, plant related parameters were determined. For mi-

Table 2: Experimental setup. Mini-lysimeters (11 cm diameter, 40 cm high) filled with a control soil or soil with different amendments were placed in the greenhouse at ambient temperature for 8 weeks

Tabelle 2: Aufbau des Versuchs. Mini Lysimeter (Durchmesser 11 cm, Höhe 40 cm) gefüllt mit Kontrollboden oder mit Böden mit verschiedener Düngemischung wurden im Glashaus bei Umgebungstemperatur 8 Wochen inkubiert

Control (soil only)	
S – Sludge	Sludge + vermiculite (**SV**)
SA1 – Sludge + ash (7.75 g; 8.2 t ha^{-1})	SA1 + vermiculite (**SA1V**)
SA3 – Sludge + ash (23.3 g; 24.7 t ha^{-1})	SA3 + vermiculite (**SA3V**)

Note: Vermiculite was applied at rate of 23.3 g per lysimeter (24.6 t ha^{-1}). All treatments received 91.2 g sludge (96 t ha^{-1})

Table 1: Heavy metal contents of the ash used, and limit values for heavy metals in ashes and sludges intended for agricultural use (in mg kg^{-1} dry matter)

Tabelle 1: Schwermetallgehalte der verwendeten Asche und Grenzwerte für Schwermetalle in Aschen und Schlämmen für die landwirtschaftliche Nutzung

	Pb	Cd	Cr	Cu	Ni	Hg	Zn
Grate ash from incineration plant Kufstein	12	1.39	112	92	46	0	344
Limit value for heavy metals in wood ash intended for use in forestry and agriculture[1]	100	8	250	250	100	–	1.50
Limit values for heavy metals in anaerobic sludges (EU–VO 2092/91)	45	0.7	70	70	25	0.4	200

[1] (HOLZNER and OBERNBERGER, 1998)

crobial and molecular analyses, the top 20 cm of the soil were sampled and sieved with a 2 mm sieve. One part was stored at 4 °C until further analyses, and one part was stored at –18 °C for later molecular biological analyses.

2.3 pH and electrical conductivity

The pH was measured in a soil:0.01 M $CaCl_2$ suspension (1:2.5) with a Metrohm 744 pH meter. Electrical conductivity was determined in a soil:water suspension (1:2.5) with a LF 330 (WTW).

2.4 Soil microbiology and microbial communities

Soil basal respiration was measured as CO_2 evolution from moist (60 % WHC) soil samples at 22 °C, using a continuous flow infrared gas analysis (IRGA) (HEINEMEYER et al., 1989). Microbial biomass carbon (C_{mic}) was determined by substrate–induced respiration (SIR) after the addition of 1 % glucose (ANDERSON and DOMSCH, 1978), using the IRGA as above. The metabolic quotient (qCO_2; mg CO_2-C g^{-1} C_{mic} h^{-1}) was calculated from the basal respiration and microbial biomass.

DNA from the soils that had not received vermiculite was extracted from bulked samples using a procedure described by FRANKE-WHITTLE et al. (2005). A Promega Wizard DNA clean up kit (Madison, Wisconsin, USA) was used to purify DNA, according to the instructions of the manufacturer. Extracted DNA was subjected to electrophoresis in a 1 % agarose gel in 1 X TAE buffer, and DNA concentration was determined by fluorescence using a PicoGreen® dsDNA quantitation kit (Molecular Probes Inc., Oregon, USA) and a fmax Fluorescence Microplate Reader (Molecular Devices, CA, USA), as described by the manufacturer.

For PCR-DGGE, extracted DNA was amplified in a PCR thermocycler (PCR Express, ThermoHybaid) using a nested PCR approach. Each PCR mixture contained 0.2 µM of each primer, 0.025 U l^{-1} Bio Therm ^{TM} DNA polymerase (GeneCraft, Münster, Germany), 1 X DNA polymerase buffer, 0.1 µg $µl^{-1}$ bovine serum albumin (BSA), 4 % (v/v) dimethylsulfoxide (DMSO), 0.2 mM dNTP-Mix and 2.5 mM $MgCl_2$ in a final volume of 24 µl. One µl of extracted DNA was added as template. To amplify total bacterial communities, the universal 16S rDNA primers 63f (MARCHESI et al., 1998) and 1378r (HEUER et al., 1997) were used. The PCR included an initial 3 min denaturation at 94 °C and was followed by 30 cycles of 1 min at 94 °C, 1 min at 62 °C and 2 min at 72 °C. Amplification was completed with a final extension step at 72 °C for 10 min. PCR products from the first amplification were used as template for a second PCR performed using the primer pair 338fGC and 518r (MUYZER et al., 1993). The PCR program for these primers differed from the one described above by using an annealing step of 30 sec at 56 °C.

DGGE was performed with the Bio-Rad DCode System. The PCR products (2 µl) were loaded onto 7 % (w/v) polyacrylamide gels containing a linear denaturing gradient ranging from 40 % to 60 % (100 % denaturant corresponds to 7 M urea plus 40 % (v/v) of deionized formamide) and were run in a 0.5 X TAE buffer at 60 °C at a constant voltage of 70 V for 16 h. After electrophoresis, gels were silver stained (SANGUINETTI et al., 1994) using an automated gel stainer (Amersham Pharmacia, Germany), photographed and air dried for storage. DGGE banding patterns were normalized and analysed using the GelCompar II software package, version 4.0 (Applied Maths, Ghent, Belgium). Calculation of the pair-wise similarities was based on the Dice correlation coefficient; dendrograms were created using the algorithm of WARD (1963).

The soils were further analysed with the COMPOCHIP microarray (FRANKE-WHITTLE et al., 2005). For these analyses, fluorescence labelling of target DNA, hybridization, scanning and image analysis was conducted as described by FRANKE-WHITTLE et al. (2005). Multivariate analysis was applied to signal-to-noise ratio data, using Canoco 4.5 (TER BRAAK and SMILAUER, 2002). ANOVA was applied to analyse the dispersion of the samples along the axes.

2.5 Plant biomass and nitrate/ammonium determination

Plants were cut one cm above the soil surface and dried over night at 80 °C for one wk to determine the dry mass. Plant and soil C and N were analysed with a CHN-Analyser (Leco TruSpec Macro CHN, St Joseph,. MI, USA). Nitrate and ammonium in the leachate was measured according to INSAM and MERSCHAK (1997).

Cress germination and seedling growth was tested. As a control we used Frühstorfer Einheitserde (a standard soil). Test substrates (sludge-ash mixtures) were admixed to the soil at an amount of 15 and 30 % (w/w) and placed in Neubauer dishes and sewn with cress (*Lepidium sativum*). Watering

occurred daily, and dishes were kept in the dark for 2 d. The germination rate was measured after 2, 3 and 10 d.

2.6 Statistics

The experiment was set up in 28 lysimeters, four replicates for each treatment. A one-way ANOVA (SPSS 11.5) was performed for all analyses (n = 4) followed by a Tukey B post Hoc test.

3 Results

3.1 Soil chemical parameters

Electrical conductivity was increased by sludge amendment and further increased by ash amendment. The application of vermiculite increased the electrical conductivity of soil samples. Fertilization with sludge alone had no effect on the soil pH. However, the pH increased from 4.9 ± 0.03 to 6.0 ± 0.1 (SA1) and to 6.8 ± 0.12 (SA3) when ash was added. Vermiculite had no significant effect on pH (Table 3).

The organic C contents of the amended soils were slightly higher than those of the control; the difference was only significant, however, for the SA3 samples. All amended samples had higher N contents than the control, and no differences among the amended samples were found. The C/N ratio was higher in the control than in the other treatments.

3.2 Basal respiration and microbial biomass

Sludge and ash amendments in most cases had no effect on microbial biomass and respiration. However, the application of a combination of sludge with a triple amount of ash (SA3) resulted in a significantly increased microbial bio-

Table 3: Soil pH, electrical conductivity and soil C and N contents
Tabelle 3: pH-Wert, elektrische Leitfähigkeit und C- und N-Konzentration der Böden

	Electrical conductivity (µS cm $^{-1}$)	pH	C_{org} [%]	Total soil N [%]	Soil C/N ratio
Control	19 ± 4.8 a	4.8 ± 0.0 a	0.99 ± 0.03 a	0.054 ± 0.006 a	18.4 ± 2.3 b
only sludge	354 ± 24 b	4.9 ± 0.0 a	1.04 ± 0.02 a	0.079 ± 0.003 b	13.2 ± 0.7 ab
SV	591 ± 73 d	4.9 ± 0.2 a	1.10 ± 0.08 ab	0.087 ± 0.010 b	12.8 ± 1.8 a
SA1	408 ± 39 b	6.0 ± 0.1 b	1.11 ± 0.08 ab	0.079 ± 0.012 b	14.2 ± 2.3 ab
SA1V	531 ± 36 cd	6.1 ± 0.1 b	1.13 ± 0.06 ab	0.083 ± 0.006 b	13.6 ± 0.3 ab
SA3	456 ± 15 c	6.8 ± 0.1 c	1.19 ± 0.05 ab	0.080 ± 0.016 b	15.3 ± 3.1 ab
SA3V	543 ± 38 cd	7.0 ± 0.1 c	1.12 ± 0.04 b	0.079 ± 0.008 b	14.3 ± 1.8 ab

Different letters in a column indicate statistically significant differences ($p < 0.05$). Abbreviations see Table 2

Table 4: Basal respiration, microbial biomass and ecophysiological quotients of lysimeter soils treated with sludge, and sludge, ash and vermiculite combinations
Tabelle 4: Basalatmung, mikrobielle Biomasse und ökophysiologischer Quotient der Lysimeter-Böden, behandelt mit Gärrest sowie mit Gärrest-, Asche- und Vermiculit-Gemischen

	Basal respiration (µg CO_2 g^{-1} soil h^{-1})	Microbial biomass (µg C g^{-1} soil)	Metabolic quotient (mg CO_2-C g^{-1} C_{mic}^{-1} h^{-1})	Percent C_{mic} in C_{org}
Control	0.20 ± 0.08 a	21 ± 2.3 a	2.6 ± 1.29 ab	0.49 ± 0.04 a
only sludge	0.21 ± 0.03 a	24 ± 1.7 a	2.4 ± 0.24 ab	0.22 ± 0.03 a
SV	0.21 ± 0.06 a	29 ± 4.5 a	2.0 ± 0.36 a	0.47 ± 0.09 a
SA1	0.33 ± 0.01 a	32 ± 2.6 a	2.8 ± 0.11 ab	0.23 ± 0.02 a
SA1V	0.30 ± 0.03 a	32 ± 3.0 a	2.5 ± 0.39 ab	0.27 ± 0.05 a
SA3	0.84 ± 0.31 b	58 ± 6.4 b	4.0 ± 1.73 b	0.29 ± 0.01 b
SA3V	0.60 ± 0.20 ab	53 ± 8.3 b	3.1 ± 0.78 ab	0.29 ± 0.05 b

Different letters in a column indicate statistically significant differences ($p < 0.05$). Abbreviations see Table 2

mass and basal respiration (SA3 and SA3V). The metabolic quotient was not changed significantly by any of the treatments (Table 4).

3.3 Microbial community

PCR-DGGE was able to differentiate the microbial communities of the differently treated soils, resulting in the formation of two main clusters (Figure 1). Control samples clustered together in one group, while all samples that had received a sludge amendment clustered together in another group that displayed only low similarity to the control samples. Within this second cluster, the ash amended soils again formed a sub-cluster (84 % similarity), while the samples that had been amended with different amounts of ash (SA1 and SA3) could be differentiated from each other. Samples amended with sludge and ash showed a high similarity between the replicates (with up to 95.2 % similarity for the SA3 samples), while the overall variability between replicates of the SA1 and SA3 samples was lower than that of the replicates of the sludge-only and control samples.

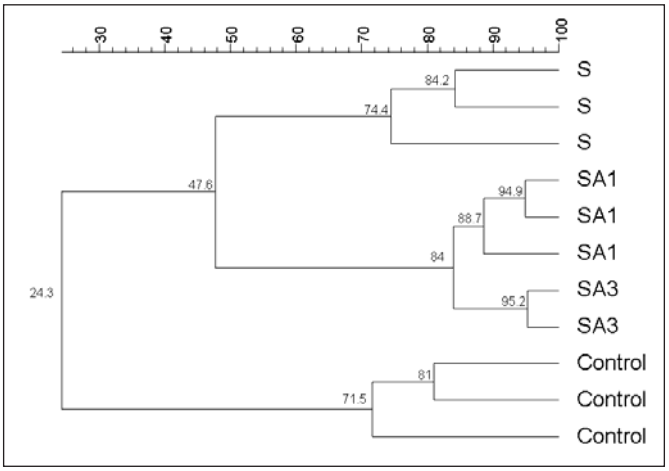

Figure 1: Cluster dendrogram of microbial communities of soils treated with different combinations of anaerobic sludge and wood ash (S … sludge only; SA1 … sludge with single amount of ash; SA3 … sludge with triple amount of ash).

Abbildung 1: Cluster-Dendrogramm der mit den unterschiedlichen Kombinationen von Gärrest und Holzasche behandelten Böden (S … nur Gärrest, SA1 … Gärrest mit einfacher Menge an Asche; SA3 … Gärrest mit dreifacher Aschemenge)

Analysis of the microarrays showed differences among the differently treated soils (Figure 2). Along principal component (PC) axis 1, untreated control soil was distinct from all soils that had received sludge. Along the PC axis 2, the samples that had received SA1 were significantly different to the samples that had received sludge only. Data variability was particularly high in the samples that had received SA1.

The PCA loading plot shows that some probes were more influential in discriminating the samples from each other. The probes KO277 and KO278, specific for *Azotobacter beijerinckii* were found to give higher signals, indicating higher numbers of *Azotobacter* in the SA1 samples. Also, the probes KO535, KO536 and KO12, specific for *Pseudomonas,* gave higher signals in the SA1 samples than in other samples. There was also a higher abundance of *Actinomyces* spp. and *Clostridium fallax/perfringens* in the sludge only samples. Ash admixture did in addition change the composition of the community, and there were several probes that gave higher signals in these amended samples, in particular, KO235 (*Streptococcus*), KO294, KO295 and KO296 (*Nitrosospira/Nitrosovibrio/Nitrosomonas*), KO464 and KO465 (*Acidobacterium*), KO319 (Low G+C), and KO261 and KO376 (*Clostridium bifermentans*).

3.4 Plant biomass and nitrate in the leachate

The sludge plus vermiculite amendment yielded the highest plant biomass, while no differences were found among the other treatments (Table 5). The percentage of leguminous plants was approximately 10 % in the control. Fertilization with sludge decreased the legume fraction to an insignificant number (data not shown). There were no differences in the C content of the plants, however, the C/N ratio was lower in all samples fertilized with sludge when compared with the control samples. The C/N ratio of the ash amended soils was slightly higher than that of the soil that had received only sludge (Table 5).

Nitrate concentrations in the leachate increased during the experiment for all amended samples (6.0–9.4 mg ml^{-1}) but not for the control (2.15 mg ml^{-1}). No differences were found among the fertilizer treatments (Table 5). Ammonium concentrations did not differ significantly among treatments and ranged from 110 to 229 µg ml^{-1}.

3.5 Cress test

When the sludge and sludge-ash combinations were applied at a rate of 30 %, the germination rate was almost zero (data not shown). At a 15 % amendment rate (Figure 3),

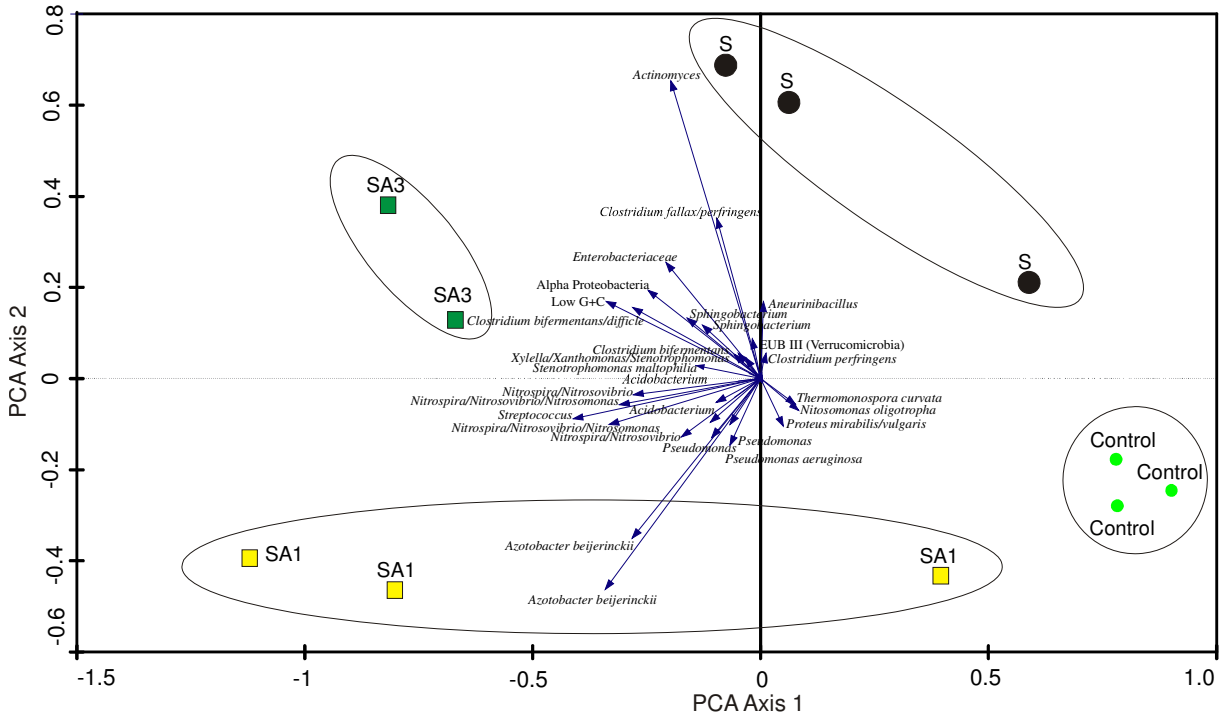

Figure 2: PCA plot of microarray data of soils treated with different combinations of anaerobic sludge and wood ash (S … sludge only; SA1 … sludge with single amount of ash; SA3 … sludge with triple amount of ash)

Abbildung 2: PCA-Plot der Microarray-Daten der mit den unterschiedlichen Kombinationen von Gärrest und Holzasche behandelten Böden (S … nur Gärrest, SA1 … Gärrest mit einfacher Menge an Asche; SA3 … Gärrest mit dreifacher Aschemenge)

Table 5: Biomass of plants, soil C/N ratios and nitrate found in the microcosm leachates of soil treated with different combinations of anaerobic sludge and wood ash (S … sludge only; SA1 … sludge with single amount of ash; SA3 … sludge with triple amount of ash)

Tabelle 5: Pflanzenbiomasse, C/N-Verhältnisse der Böden und Nitratkonzentrationen der Perkolate (Kontrolle, Behandlung mit Gärrest, Gärrest und Vermiculit, sowie Gärrest-, Asche- und Vermiculit-Gemischen)

Treatment	Plant biomass (g)	Soil C/N-ratio	Nitrate in leachate (mg ml^{-1})
Control	185 ± 27 b	8.2 ± 0.88 a	2.15 ± 0.31 a
Only sludge	109 ± 56 c	5.4 ± 0.20 c	7.8 ± 0.08 b
SV	364 ± 35 a	6.2 ± 0.23 bc	7.89 ± 0.89 b
SA1	090 ± 24 c	5.9 ± 0.074 bc	9.04 ± 2.03 b
SA1V	151 ± 77 bc	6.0 ± 0.31 b	9.79 ± 1.12 b
SA3	102 ± 15 c	6.4 ± 0.17 b	8.21 ± 1.09 b
SA3V	165 ± 13 b	6.2 ± 0.067 bc	9.35 ± 2.07 b

Different letters in a column indicate statistically significant differences ($p < 0.05$). Abbreviations see Table 2

the germination rate on day 1 was only 10–20 % of that of the control. On day 3, germination rates for the ash treatments without vermiculite were lower than for all other treatments. On day 10, however, the germination rates were almost similar for all treatments and ranged between 93 % and 98 % of the control.

4 Discussion

4.1 Soil chemistry

Fertilization with anaerobic sludge is assumed to have a similar fertilizing effect as liquid manure, increasing the nitrogen available in the soil (PÖTSCH, 2005). The high content of Ca, K and Mg in wood ash results in an immediate neutralization of acid soils upon application (CLAPHAM and

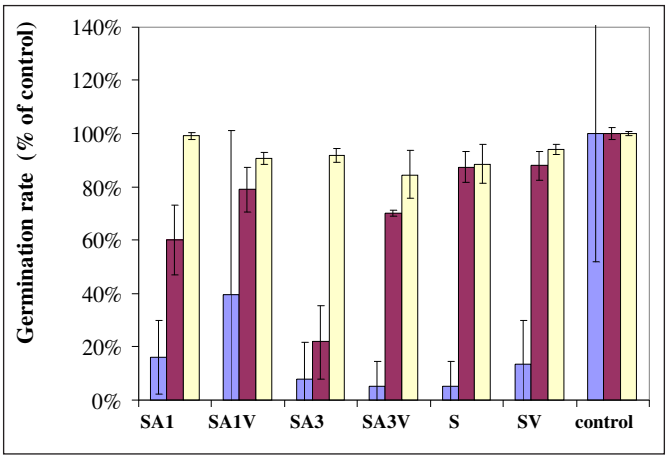

Figure 3: Germination rate of cress in control soil and in soils treated with 15 % anaerobic sludge, mixtures of sludge with ash at different rates and with or without the addition of vermiculite (details see Table 2). Measurements were made after 2, 3 and 10 d of incubation

Abbildung 3: Keimrate der Kresse im Kontrollboden und in Böden behandelt mit 15 % Gärrest, Gärrest-Asche-Mischungen mit und ohne Vermiculit-Zugabe nach 2, 3 und 10 Tagen Inkubation

ZIBILSKE, 1992; SAARSALMI et al., 2006). Thus, wood ash application can help to reduce the solubility of Al and heavy metals in soils (CLAPHAM and ZIBILSKE, 1992; MOILANEN et al. 2002; STEENARI et al., 1999; ZIMMERMANN and FREY, 2002,). Despite concerns, LEVULA et al. (2000) found no effects on plant heavy metal concentrations after ash fertilization.

The increase in electrical conductivity of a soil is often an argument against the use of sludge (CHANG et al., 1990) and wood ash as an amendment to soils. In our study, however, the limit value of 750 µS cm^{-1} (WITHERS et al., 1978) was almost never reached upon amendment with these materials (except for SA1V, 805 µS cm^{-1}). We hypothesise that leaching of nutrients decreased with time and that vermiculite further increased the cation exchange capacity of soils and thus retaining the nutrients for a longer time.

The increase of C_{org} due to ash amendment was unexpected but may be explained by the residual recalcitrant C in the ash. However, it is known that ash amendments to soils increase dissolved organic C (DOC) (LUDWIG et al., 2000; PERKIÖMÄKI and FRITZE, 2002). DOC in turn may serve as a substrate for microorganisms which in the long term would lead to a net consumption of C_{org}. The increase of C_{org} due to sludge addition is attributed to components of higher molecular weight (lignin, cellulose) that usually remain undegraded during anaerobic digestion.

4.2 Soil microbiology and microbial communities

Microbial biomass was measured in a range from 21 to 58 µg Cmic g^{-1}soil, low values when compared to other mesocosm studies (INSAM and MERSCHAK, 1997). This was probably due to the poor nutritional status of the soil used. In contrast to other studies (INSAM and MERSCHAK, 1997) where significant increases in microbial biomass and respiration were found, we found such effects only in a few cases. The positive effect on microbial biomass and respiration found with the triple addition of wood ash supports the results from other studies (BAATH et al., 1995; DEMEYER et al., 2001; PERKIÖMÄKI and FRITZE 2002, ZIMMERMANN and FREY, 2002). There are four possible reasons for these findings, (1) wood ash increases the soil pH and thus enhances the growth of neutrophilic microorganisms, (2) the higher pH increases the fraction of DOC which is the main resource for microbial growth, (3) the composition of the DOC is shifted from acids to carbohydrates, and (4) the composition of the microbial community may be altered (JOKINEN et al., 2006).

The metabolic quotient (qCO_2) is often used as an indicator for stress, and the qCO_2 of agricultural soils usually ranges from 0.2 to 3.8 mg CO_2-C $g^{-1}C_{mic}$ h^{-1} (BÖHM, 2005). Ash fertilization increases basal respiration and microbial biomass to a similar extent, and, in a study by ZIMMERMANN and FREY (2002), the qCO_2 was found to remain unaffected. We also did not find any effect of the amendments on the qCO_2, indicating that none of the treatments imposed a particular stress on the microbiota.

The application of DGGE and microarray analysis to DNAs extracted from the different soils investigated in this study revealed differences in the microbial communities of the soils amended with sludge and ash. Cluster analysis of fingerprinting patterns clearly differentiated control samples from the amended soils. Additionally, the soils treated with ash could be differentiated from the samples amended with sludge only. Despite some variation among replicate samples, PCA of the microarray data also showed the control soil samples to be different to the other samples. Specific populations were identified to be responsible for the discrimination among sample types, and it is likely that certain microorganisms were favoured as a result of the treatment applied. An impact of organic fertilization on the composition of the soil microflora has been shown previously by FROSTEGARD et al. (1997) who proposed three synergistic effects as a result of organic fertilization, 1) liquid manure can close pores and create small anaerobic sites (ma-

nure hot spots) where anaerobic and facultative anaerobic microorganisms are favoured, 2) high N-loads might eliminate microorganisms that are sensitive to high N concentrations and 3) new C sources, mainly hard to degrade ones, are being introduced. These effects could favour specialists. The introduction of non-indigenous microorganisms with the manure may also occur. PEACOCK et al. (2001) found by studying PLFA patterns that fertilization with cattle manure favoured Gram-negative bacteria. This outcome was supported by a study undertaken by LARKIN et al. (2006) who not only detected an increase in bacterial biomass and activity after manure amendment of soil, but also an increase in fatty acid methyl ester (FAME) biomarkers for Gram-negative organisms. The application of fly ash has also been shown to have a beneficial effect on Gram-negative bacteria (SCHUTTER and FUHRMAN, 2001).

In this study, high signals for the Gram-negative bacterium *Azotobacter beijerinckii* probe were obtained from the soil amended with sludge and a single dose of wood ash. This organism is known to be capable of nitrogen fixation, and was initially isolated from activated sludges (SKERMAN et al., 1980). Lower levels of this organism were also found in the soil amended with sludge and a triple dose of wood ash, but this organism was not detected in the soil amended with sludge alone. It is possible that *Azotobacter beijerinckii* prefers a more alkaline environment for growth.

The probes specific for *Pseudomonas* were also found to give higher signals in the SA1 samples than in other samples. It would thus appear that the mildly alkaline conditions along with an increase in available C provided by the ash and the sludge together were favourable for *Pseudomonas* growth. The probe targeting Low G+C bacteria was found to give higher signals in the SA1 and SA3 samples. This supports the results of the DGGE whereby the SA1 and SA3 samples could be differentiated from all other treatments by some distinctive bands in the upper part of the gel where the denaturing gradient is low and organisms containing a low G+C content are located. Several authors (INNEREBNER et al., 2006; MARSCHNER et al., 2003; SUN et al. 2004) used PCR-DGGE to reveal a shift in bacterial community composition caused by the amendment of manure in long-term fertilizer experiments. This outcome has been attributed to the growth promoting effect of manure on indigenous *Bacillus sp.* in the soils (CHU et al., 2007). Thus, our results correlate with those of previous studies and clearly indicate that the use of ash and sludge change microbial communities in amended soils.

Actinobacteria are often associated with plant disease suppressivity of soils (VAN BRUGGEN and SEMENOV, 2000). DANON et al. (2008) have found that in curing composts the proportion of *Actinobacteria* increased and this was associated with the disappearance of phytotoxicity. According to Jaques Fuchs (personal communication) the incidence of plant diseases decreased when composts had undergone anaerobic phases. The association of *Actinomyces* with anaerobic sludge treatment might thus indicate beneficial effects in terms of soil health.

4.3 Plant biomass and nitrate in the leachate

Anaerobic sludge has been found to have fertilizing properties similar to mineral NPK fertilizers and liquid manure (PÖTSCH, 2005). Nitrogen-rich fertilizers are known to decrease the fraction of leguminous plants (PÖTSCH, 2005). This was also observed in our study (data not shown).

Several studies have shown positive effects of ash on plant growth, e.g. for trees (MOILANEN et al., 2002), or for agricultural and horticultural plants (CLAPHAM and ZIBILSKE, 1992; ERICH and OHNO, 1992). However, due to the elemental composition of ashes, beneficial effects have only been demonstrated in combination with N fertilizers (ERIKSON, 1998). Sludges are efficient N fertilizers, and thus the combination with wood ash should have increased plant growth as has been shown for corn (WENZEL and ZIVKOVIC, 2006). Several reasons may explain the low plant yield that was observed in our study after ash and/or sludge application. In our study, however, with the exception of the SV sample, no significant differences in plant biomass production were seen with the different applications.

Soils with an electrical conductivity from 250 to 750 µS cm^{-1} are considered to be saline (WITHERS et al., 1978). With the high sludge application rates, and particularly in combination with ash, a K^+ surplus may have retarded the plant growth due to a cation imbalance (STEENARI et al., 1999). Firstly, we used a grass mixture that did not have high N requirements and we may thus have applied too high an amount of sludge. Nutrient imbalances due to elevated pH are also possible (reduced availabilities of P, Mg or Fe) (SCHILLING et al., 2000). Another explanation for retarded plant growth could be the production of phytotoxic intermediates (eg. hippuric, salicylic or benzoic acid) from microbial degradation of the liquid manure (BEER and SUNTHEIM, 2003). In future experiments, it would be better to use plants with a higher nitrogen demand (e.g. corn), or to reduce the amount of sludges applied. The positive effect of vermiculite on plant biomass is

an indication of some salt stress and indicates that a combination of sludges, ash and vermiculite may have the best fertilizer properties.

Anaerobic sludge usually contains higher amounts of ammonium (FUCHS et al., 2004) than liquid manure does. This is due to the C losses during anaerobic digestion and the resulting narrowing of the C/N ratio (AMON and DÖHLER, 2005). With increasing pH, the dissociation balance shifts from NH_4^+ to NH_3. Under aerobic conditions in the soil, ammonium is rapidly nitrified, which results in increasing nitrate contents in the leachate. STAUFFER and SPIESS (2005) found nitrate concentrations of 100 mg $NO_3^- l^{-1}$ in the leachate of a grassland experiment. In other studies however, only 1.35 mg NO_3-l^{-1} was found in the leachate of a grassland field trial after fertilizing with anaerobic sludge (120 kg N ha^{-1}) (PÖTSCH, 2005). This discrepancy emphasises a need for field trials. The high nitrate loads in our experiments are further attributed to the low N demand of the grass mixture used, and the high fertilization rate (> 180 kg N_{tot} ha-l^{-1}). While the addition of wood ash reduced nitrate leaching from soils that had not received an organic input (KUBA et al., 2008) it did not act in the same way in our experiment with the combined addition of ash and sludge. The lack of an effect of vermiculite for nitrate retention may be explained by the high amount of other cations from ash and sludge that occupied the binding sites. For future experiments and applications, a reduction in the amount of sludge and ash would be advisable.

4.4 Cress tests

Retarded germination rates of the cress seedlings may have been caused by the same reasons as discussed above for the reduced plant biomass yields in the lysimeter tests. The additional reduction of germination by ash amendment is attributed to the high ion concentration, and ammonia liberation due to the elevated pH. Soil ion concentration and ammonia liberation was reduced by vermiculite, which explains the higher germination rates found when vermiculite was added.

5 Conclusions

The aim of this study was to investigate if the combined use of ash and sludge would change soil microbiological properties and improve plant growth, and if high doses of these materials could create problems in terms of nitrate leaching. We conclude that anaerobic sludge or its combination with ash improves several soil microbiological parameters and changes the microbial community. Even at the high application rates tested here, the sludges are unlikely to endanger the groundwater by nitrate leaching. The rates applied in this study exceeded plant needs and the high salt contents may have adversely affected plant growth. These effects, however, were alleviated by vermiculite. For future experiments on combination effects, as well as for field applications, we recommend using lower amounts of sludge. Vermiculite seems to be a promising additive to fertilizers from sludge and ashes.

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