ELSEVIER

FEMS Microbiology Ecology 44 (2003) 27–33

FEMS MICROBIOLOGY Ecology

www.fems-microbiology.org

Does simulated acid rain increase the leaching of cadmium from wood ash to toxic levels to coniferous forest humus microbes?

Jonna Perkiömäki, Hannu Fritze *

Finnish Forest Research Institute, Vantaa Research Center, PO Box 18, 01301 Vantaa, Finland

Received 21 August 2002; received in revised form 24 October 2002; accepted 4 November 2002

First published online 7 December 2002

Abstract

Wood ash contains Cd in concentrations not permitted for fertilization use in agriculture (>3 mg kg^{-1}). It has been shown that spiking ash with Cd to concentrations of 1000 mg kg^{-1} induced no further changes in humus microbial activity and community structure as ash alone. To accelerate the weathering process and thus to liberate the spiked Cd from the ash, three treatments – wood ash (A), Cd spiked wood ash (ACd, 1000 mg Cd kg^{-1} ash), both applied at a fertilization rate of 5000 kg ha^{-1}, together with a control (C) – were performed in microcosms and incubated in field condition under two types of irrigation – water and simulated acid rain. During the incubation period of one growing season the simulated acid rain plots received a sulfur load of 3.64 g S m^{-2}, which was 15 times more than the S deposition on the water irrigated plots. The treatments resulted in a mean Cd increase of the humus from 0.23 mg kg^{-1} of the C treatment to 0.52 and 39.5 mg kg^{-1} of the A and ACd treatments, respectively. The irrigation had no further effect on the result. The microbial activity, measured as soil basal respiration, and the microbial community structure, measured as humus phospholipid fatty acid and 16S and 18S polymerase chain reaction/denaturing gradient gel electrophoresis patterns, changed only due to the ash (A and ACd treatments) fertilization irrespective of the irrigation. The bacterial biosensor, emitting light in the presence of bioavailable Cd, did not react to any of the treatments. This result shows that Cd in ash was not leached into the humus due to increased deposition of acidified rain.

Keywords: Cadmium; Denaturing gradient gel electrophoresis profiling; Humus; Multivariate analysis; Phospholipid fatty acid; Wood ash

1. Introduction

During the last decade, there has been increasing interest in using logging residues for bioenergy in Scandinavia. The use of such residues for energy production generates large amounts of wood ash. Intensive harvesting increases nutrient export and soil acidification [1] and therefore it has been suggested to recycle the nutrients contained in the wood ash. Due to improved technology in cleaning exhausts from power plants the resulting fly ash, originating from bark or stems, has become enriched with heavy metals. The use of this wood ash in forest fertilization has therefore been questioned on the ground of the cadmium (Cd) concentration of the ash, which varies between 1 and 30 mg kg^{-1} ash [2], thereby exceeding the level allowed for fertilizers (3 mg kg^{-1}) used in agriculture. Therefore the impact of this ash on the forest ecosystem has to be investigated.

Humus microbes, being responsible for the mineralization of organic material, play an important role in the functioning of the forest ecosystem. Cd has been shown to inhibit forest soil microbial activity and to change the microbial community structure already at very low levels with the range from 1 to 5 mg Cd kg^{-1} humus being the lowest toxicity inducing values reported for heavy metals in general [3]. In a recent microcosm study we could show that wood ash added onto the humus layer as top dressing and artificially enriched with Cd up to a level of 1000 mg kg^{-1} ash induced the same changes to the humus microflora than unspiked ash addition itself. Ash thus protected the microflora from the harmful effects of Cd since the same amount of Cd added onto the humus layer without ash decreased the soil respiration and changed the bacterial community structure [4]. Furthermore Cd was bioavailable only when ash was not added to the microcosm [5]. This is due to the fact that ash fertilization increases

* Corresponding author. Tel.: +358 (9) 85705407; Fax: +358 (9) 8572575.
E-mail address: hannu.fritze@metla.fi (H. Fritze).

0168-6496/02/$22.00
PII: S0168-6496(02)00457-9

the pH of the humus layer [6], which in turn affects the solubility of Cd.

Wood ash dissolves more rapidly when treated with acidified water [7]. As acidic deposition is still a threat to the terrestrial environment [8] this could affect the liberation of Cd from the ash into the soil. In general nothing is known about the effect of simulated acid rain (SAR) on the microbial function and community structure of coniferous forest humus fertilized with wood ash. The aims of this study were therefore to elucidate the effects of wood ash fertilization under water and acidified water irrigation and to determine the effect of Cd spiked into the ash in response to the treatments. In order to determine treatment induced microbial responses basal respiration, microbial community structure and Cd bioavailability were determined.

2. Materials and methods

2.1. Microcosms and treatments

Coniferous forest humus was collected in May 2001 from a Norway spruce (*Picea abies*) stand growing on a *Myrtillus* site type in southern Finland. The humus was passed through a 2.8-mm sieve by hand and stored at 4°C. The physico-chemical properties were determined from air-dried samples prepared within two weeks after sieving. The humus was characterized to have a pH value of 4.1, a cation-exchange capacity of 30.2 cmol kg^{-1}, a base saturation of 25.1% and a C/N ratio of 18 (see Section 2.2). The humus was kept at 4°C for a total of 4 weeks before the experiment was started.

All together 120 pots were filled with 110 g of humus. Randomly chosen sets of four pots were either treated with wood ash (A), Cd spiked wood ash (ACd) or left untreated (C). For the ACd treatment wood ash was spiked with CdO to give a Cd concentration of 1000 mg kg^{-1}ash. The Cd was mixed into the ash by rotation over night before determining the Cd concentration of the mixture (Table 1). To mimic a dose of 5000 kg ash ha^{-1}, 3.6 g of ash was added to the humus as top dressing. The microcosms were then placed outdoors in a field experiment consisting of, respectively, five treatment plots receiving either water or SAR (pH 3, sulfuric acid). Always four replicate treatment microcosms were placed on one field plot on 18 June. This experimental area is situated near the Kevo Subarctic Research Station (69°45′N, 27°01′E) in northern Finland where the growing season is 110–125 days. The irrigation in the field was started on 15 June and ended on 13 September. During this period the plots were irrigated 40 times and the SAR plots received a sulfur load of 3.64 g S m^{-2}, which was 15 times more than the S deposition on the water irrigated plots.

The 120 microcosms were destructively sampled on 22 September and brought to the laboratory within three days. Of the four treatment replicates per irrigation plot always two were combined to give a total of 60 samples. From these the respiration and biosensor measurements were taken. Finally all treatment replicates per irrigation plot were combined for the microbial community structure analyses and the physico-chemical measurements ($n = 30$).

2.2. Chemical analyses

The dry matter weight (d.m.) was determined by drying duplicate subsamples at 105°C overnight. Total organic carbon and nitrogen content were determined by dry combustion (Leco CHN-600). Cd and other total elemental concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, ARL 3580) after wet digestion and extraction with HNO_3–H_2O_2. In the case of original humus the extractable elements were eluted 0.1 M $BaCl_2$ before measuring with ICP-AES. The humus pH was measured in a water suspension (1:15, w/v). See Table 1 for the characterization of the humus and ash used.

2.3. Microbial analyses

The basal respiration rate was measured as the amount of CO_2–C evolved in 25 h [9]. Fresh humus samples, equalling 2 g dry weight, were used in the analyses.

The phospholipid fatty acids (PLFAs) were extracted and analyzed from 1 g fresh weight of humus [10,11]. The total amount of PLFAs ($PLFA_{tot}$) was used to indicate the total microbial biomass, and the sum of PLFAs considered to be predominantly of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0) was chosen as an index of

Table 1
Characterization of the humus and ash used in this experiment

	Humus (mg kg^{-1}; extractable)	Humus (mg kg^{-1}; total)	Ash (mg kg^{-1}; total)
K	258	1362	25611
Ca	1180	2412	286025
Mg	118	2568	17211
Na	16.3	180	6790
B	ND	3.911	218
Mn	43.5	156.5	8238
P	7.88	782.2	8427
S	–	836.1	14022
Al	952	18270	14767
Fe	129	16610	7647
Cd	ND	0.2342	10[a]
Cr	–	22.12	58
Cu	ND	24.68	58
Ni	–	8.311	60
Pb	–	34.04	57
Zn	10.2	45.09	2420

ND = not detected.
– = not analyzed.
[a]The Cd content of the spiked ash was 930 mg kg^{-1}.

the bacterial biomass ($PLFA_{bact}$) [12]. The amount of 18:2ω6 was used as an indicator of fungal biomass ($PLFA_{fung}$), since 18:2ω6 is suggested to be mainly of fungal origin in soil, and it is known to correlate with the amount of ergosterol [12].

Humus DNA extraction for PCR/DGGE (polymerase chain reaction/denaturing gradient gel electrophoresis) using general bacterial 16S [13] and fungal 18S rDNA primers [14] followed the protocol of Pennanen et al. [15] using 300 mg of fresh humus weighted into Bead Solution tubes (Ultraclean Soil DNA Isolation Kit, Mo Bio Laboratories Inc.). Consult Heuer et al. [13] for the PCR/DGGE conditions when using the bacterial F984+R1378 primer pair. In contrast to Heuer et al. [13] the denaturing gradient was from 35 to 60% of denaturant and the DGGE was performed in 1×TAE buffer at 58°C at a constant voltage of 150 V for 5 h. The fungal PCR/DGGE was performed with the primer pair FF390+FR1 [14], according to the descriptions given by Vainio and Hantula [14] and Pennanen et al. [15]. The wells of the DGGE gels were loaded with approximately the same amount of DNA. The DNA fragments were visualized by SYBRGreen I (FMC BioProducts) staining under UV light and photographed with a AlphaDigiDoc™ camera system.

The bioavailability of Cd in the humus samples after the treatments was determined by using the *Bacillus subtilis* strain BR151 [16] containing the Cd sensor plasmid pTOO24 [17] to control the expression of firefly luciferase. This biosensor emits light specifically in the presence of Cd. Air-dried humus samples of 2.5 g were used for the analyses. To obtain the relationship between bioluminescence and Cd concentration dilutions from a 10 mM $CdCl_2$ solution were prepared in Milli-Q (Millipore, Bedford, MA, USA) purified water to reach final Cd concentrations between 1 pM and 1 mM. The procedure was performed as described in detail by Fritze et al. [5]. Induction coefficients were calculated with different amounts of Cd as follows: induction coefficient $I = L_i/L_b$, where L_i is the light emitted by an induced sample (containing Cd) and L_b is the light emitted by the non-induced sample (containing Milli-Q water). The induction coefficient was then plotted against increasing Cd concentration, resulting in a typical standard curve for metal biosensors having a maximum I value (I_{max}) at a certain metal concentration where after the I value decreases again with increasing metal concentration. The same procedure was performed for the undiluted water extracts of the humus samples from the treatments. The light emission was converted to Cd concentrations using the linear part of the standard curve before reaching I_{max}.

2.4. Statistical analyses

The results were presented per d.m. of humus. The 38 identified individual PLFAs were expressed as mole percentage (mol% = area% of a single PLFA from the area sum of all identified PLFAs). The mol% values from the PLFA were standardized by dividing by the standard deviation (correlation matrix) before being subjected to principal component analysis (PCA). Two ACd treatments, one under water and one under SAR had to be removed from the PLFA data as outliers due to contamination in the laboratory. All the results, including the scores of the multivariate analysis, were subjected to analysis of variance (ANOVA) followed by the LSD (least significant difference) test for comparison of means. Two main effects and their interactions were tested in the ANOVA. The main effects were the treatments (C, A, ACd) and irrigation (water, SAR). If irrigation plot treatment replicates existed (respiration, biosensor), their mean was used and the final n for the statistical analyses was thus 30, except for the PLFA data where n was 28 (see above). In the case of the biosensor data a Kruskal–Wallis non-parametric ANOVA followed by a mean ranks test had to be performed due to the uneven distribution of positive results between treatments and irrigation (see Section 3). Pearson

Table 2
Treatment related humus physico-chemical and microbial variables (mean ± SE)

	Irrigation					
	Water			SAR		
Variable*	C**	A	ACd	C	A	ACd
pH	$5.0^{a} \pm 0.05$	$7.6^{b} \pm 0.08$	$7.6^{b} \pm 0.05$	$4.8^{a} \pm 0.03$	$7.5^{b} \pm 0.04$	$7.5^{b} \pm 0.05$
C (% of d.m.)	16.2 ± 0.43	15.1 ± 0.62	16.9 ± 0.51	16.9 ± 0.99	15.5 ± 0.37	16.2 ± 0.79
N (% of d.m.)	$0.87^{a} \pm 0.02$	$0.77^{b} \pm 0.03$	$0.86^{ab} \pm 0.03$	$0.89^{a} \pm 0.05$	$0.78^{b} \pm 0.02$	$0.81^{ab} \pm 0.03$
Ca (g kg^{-1})	$2.43^{a} \pm 0.06$	$18.8^{b} \pm 0.25$	$18.7^{b} \pm 0.45$	$2.40^{a} \pm 0.08$	$18.1^{b} \pm 0.41$	$18.5^{b} \pm 0.16$
Cd (mg kg^{-1})	$0.23^{a} \pm 0.02$	$0.53^{a} \pm 0.02$	$44.9^{b} \pm 9.05$	$0.24^{a} \pm 0.02$	$0.51^{a} \pm 0.03$	$34.1^{b} \pm 2.13$
CO_2–C (μg g^{-1} h^{-1})	$2.49^{a} \pm 0.13$	$3.24^{b} \pm 0.33$	$3.41^{b} \pm 0.16$	$2.42^{a} \pm 0.08$	$3.73^{b} \pm 0.16$	$4.03^{b} \pm 0.23$
$PLFA_{tot}$ (nmol g^{-1})	934 ± 38	857 ± 34	813 ± 92	966 ± 40	842 ± 44	913 ± 71
$PLFA_{bact}$ (nmol g^{-1})	395 ± 40	354 ± 17	333 ± 40	413 ± 16	351 ± 18	378 ± 32
$PLFA_{fung}$ (nmol g^{-1})	$33.9^{a} \pm 1.3$	$27.1^{b} \pm 1.3$	$27.5^{b} \pm 3.5$	$33.6^{a} \pm 1.9$	$26.8^{b} \pm 2.1$	$30^{b} \pm 2.5$

SAR = simulated acid rain.
*Means of bold variables are significantly different between the two irrigation treatments.
**Treatment means indexed by different letters are significantly different. Two-way-anova followed by LSD-test.

correlation tests were also performed between the PCA scores and the pH.

The bacterial and fungal DGGE gel photographs were analyzed as follows. All existing bands were first identified and named (A, B, C, ...). From the bacterial and fungal DGGE 19 and 34 separate bands, respectively, could be identified. Then all sample lanes ($n = 30$) were screened for the presence (1) or absence (0) of the respective bands, ending in a data matrix having only 1 and 0. This matrix was analyzed with a non-metric multidimensional scaling (MDS) method, which considers the rank order of distances. The principle on which MDS operates is to find a graphical representation of the data in a few dimensions, the distances in the ordination of the samples reflecting the (dis)similarities between the respective DGGE patterns as closely as possible. The pairwise (dis)similarities were computed using a Jaccard coefficient designed for this kind of data. Due to the nature of MDS, and unlike PCA, no further statistics can be made for the DGGE data.

3. Results

3.1. Effect of treatment

The treatment (C, A, ACd) means for the measured variables are presented separately for the water and SAR irrigation in Table 2. According to ANOVA no interactions occurred between treatments and irrigation (see Table 3 for an example). This meant that the treatments C, A, and ACd induced similar responses when irrigating with water or SAR. Therefore the overall mean treatment values, each treatment having $n = 10$, are presented here in the text. The ash treatments increased the humus pH significantly ($P < 0.001$) from the C treatment value 4.9 to 7.5 and 7.5 for the A and ACd treatments, respectively. The treatments had no effect on the C contents of the humus but the total N decreased from 0.88% (% of d.m.) to 0.78% due to the A treatment. The N content of the ACd treatment was 0.84% and did not differ from the control (Table 2). Both ash treatments increased the total humus Ca concentration from 2.4 g kg^{-1} to 18 g kg^{-1}. The humus total Cd concentration was 0.23 mg kg^{-1} in the C treatment and 0.52 and 39.5 mg kg^{-1} in the A and ACd treatments, respectively.

The basal respiration increased significantly ($P < 0.001$) due to the ash treatments. The mean CO_2–C production of the C treatments was 2.45 μg g^{-1} h^{-1} and increased due to the A treatment to a level of 3.48 μg CO_2–C g^{-1} h^{-1} and due to the ACd treatment to 3.73 μg CO_2–C g^{-1} h^{-1}. There were no significant differences between the A and ACd treatments. All the PLFA derived microbial biomass measures decreased due to the ash treatments (A and ACd). The total microbial biomass $PLFA_{tot}$ decreased from the mean C value of 950 nmol g^{-1} to 863 nmol g^{-1} and 850 nmol g^{-1} for the ACd and A treatments, respectively. This decrease was statistically insignificant and there were no differences between the two ash treatments. The respective values for the bacterial biomass $PLFA_{bact}$ were 404 nmol g^{-1}, 355 nmol g^{-1} and 353 nmol g^{-1} for the C, ACd and A treatments. This decrease was near statistical significance ($P = 0.051$) and no difference was detected between the A and ACd treatments. The fungal biomass $PLFA_{fung}$ decreased significantly ($P < 0.01$) from the mean C value of 33.7 nmol g^{-1} to the respective ACd and A values of 28.7 nmol g^{-1} and 26.9 nmol g^{-1}. No differences between the A and ACd treatments were detected.

The induction coefficient I of 21 samples was ≤ 1 and thus no bioavailable Cd could be detected with the biosensor in these samples. Bioavailable Cd was detected from nine samples. The treatment means for A ($n = 4$) and ACd ($n = 5$) were 0.27 ± 0.11 mg Cd kg^{-1} and 0.33 ± 0.09 mg Cd kg^{-1}, respectively, and did not differ

Table 3
ANOVA for soil respiration

Source	*DF*	Mean square	*F*
Treatment (C, A, ACd)	2	9.13	23.03*
Irrigation (water, SAR)	1	0.901	4.55**
$T \times I$	2	0.338	0.203
Residual	24		

*$P < 0.001$.
**$P < 0.05$.

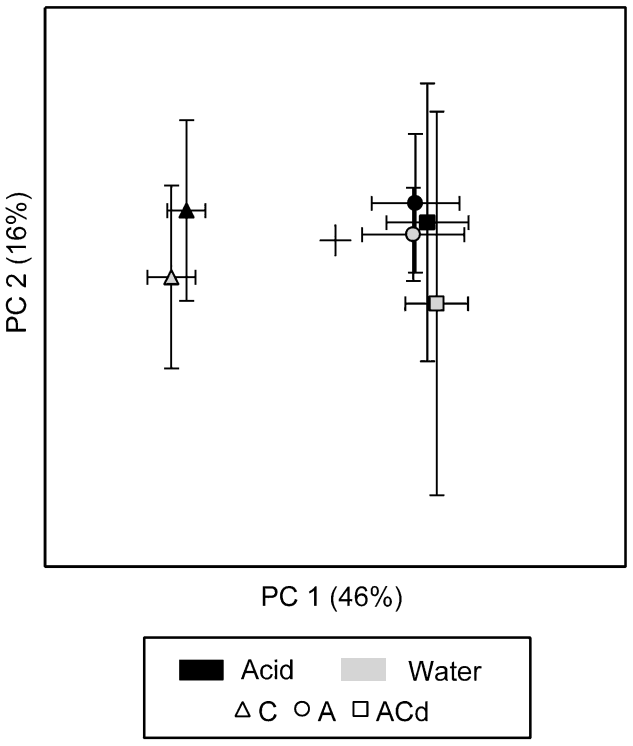

Fig. 1. PCA using the mol% of the PLFAs from irrigated humus samples. Abbreviations used: C = control, A = wood ash, ACd = cadmium spiked wood ash. Treatment means ± SD are presented.

significantly from each other ($P = 0.5$; Kruskal–Wallis ANOVA).

The mol% of the individual PLFAs was subjected to PCA, which explained 62% of the data variation. The scores of PC1 were correlated to the humus pH ($r = 0.95$, $P < 0.001$) and separated the two ash treatments from the controls (Fig. 1). No further separation of the treatments could be detected. The loadings of the individual PLFAs on the first two PC axes are presented in Table 4. PLFAs with high positive or negative loading values on PC1 contributed most to the separation of the treatments along this axis (Fig. 1). MDS analyses of the DGGE patterns verified the result obtained with the PLFAs; both ash treatments had similar bacterial or fungal DGGE patterns, which were separated from the respective controls (Fig. 2). Respectively eight and seven bands were mainly responsible for the separation of the ash treatments in the bacterial and fungal DGGE.

Table 4
Loadings of the individual PLFAs on the first two PCAs

PLFA	PC1	PC2
i14	0.2262	−0.0067
14:0	0.1421	−0.1549
i15	−0.2036	0.0393
a15	−0.0734	0.0078
C15:1	0.0825	−0.3278
15:0	0.1681	−0.0421
i16:1	−0.2189	−0.0043
C16:0	−0.1964	−0.1679
i16:0	−0.1801	0.0132
16:1w9	−0.1437	0.0382
16:1w7c	0.2288	0.072
16:1w7t	0.0963	0.2674
16:1w5	−0.2168	0.0507
16:0	0.2221	0.0282
br17	−0.0351	−0.2251
10Me16	−0.2239	−0.0245
i17	−0.2187	0.0001
a17	−0.1758	−0.16
17:1w8	0.0696	−0.3701
cy17	0.2196	−0.001
C17:1	−0.2049	−0.0977
17:0	−0.0145	−0.0518
br18	−0.2293	−0.0346
10Me17	−0.1237	−0.0671
18:2a	0.0988	−0.1413
18:2w6	−0.1128	0.11
18:1w9	−0.2131	0.126
18:1w7	0.1797	0.13
18:1	−0.1046	−0.2455
18:0	−0.0643	−0.2082
19:1a	0.2202	−0.0095
18:2b	−0.0552	−0.0068
10Me18	−0.1645	−0.1193
19:1b	0.0097	−0.3857
cy19	−0.1658	−0.0106
20:5	0.1337	−0.291
20:4	0.0141	0.1401
20:0	0.1287	−0.2956

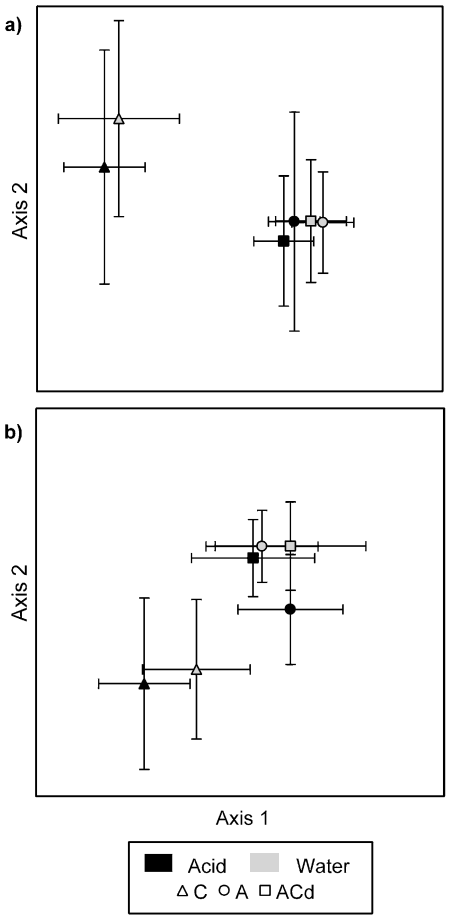

Fig. 2. Non-metric MDS ordination of the DGGE data matrix of (a) bacterial and (b) fungal rDNA fragments from irrigated humus samples. See legend of Fig. 1 for abbreviations. Treatment means ± SD are presented.

3.2. Effect of irrigation

The effect of SAR on the results was statistically verified by comparing the respective mean values of all watered ($n = 15$) to all acidified microcosms ($n = 15$). SAR decreased the humus pH of the microcosms significantly ($P < 0.05$) from a mean value of 6.7 of all watered plots to a mean pH value of 6.6. The difference between the two respective control treatments was 0.2 pH units (Table 2). Due to SAR the basal respiration increased significantly ($P < 0.05$, Table 2) from 3.05 μg CO_2–C g^{-1} h^{-1} to 3.4 μg CO_2–C g^{-1} h^{-1}. The irrigation had no effect on the humus C and N content, the total Ca and Cd concentration or the microbial biomass values $PLFA_{tot}$, $PLFA_{bact}$ and $PLFA_{fung}$. The scores of PC1 and PC2 were not significantly related to the irrigation treatment and thus no irrigation effect on the PLFA pattern could be obtained. Again the MDS analyses of the bacterial and fungal DGGE patterns verified this result. The positive biosensor data was unevenly distributed between the irrigation treatments. The SAR treatment had a mean ($n = 2$) bioavailable Cd of 0.14 ± 0.04 mg kg^{-1} and did not significantly differ

from the watering treatment mean ($n = 7$) of 0.35 ± 0.08 mg Cd kg^{-1} ($P = 0.15$; Kruskal–Wallis ANOVA).

4. Discussion

Application of wood ash onto the humus increased the pH and this was accompanied by an increase in basal respiration rate, a change in microbial PLFA pattern and a slight decrease in PLFA derived biomass values. These results thus confirm the published results [4,18–20]. In addition to the PLFA method, which is a measure of microbial community structure, also the PCR based DGGE method was used to test the treatment effects separately on the bacterial or fungal community. Both approaches showed that ash induces changes in the bacterial and fungal community. Again it could be shown that wood ash spiked with cadmium to a level of 1000 mg Cd kg^{-1} ash induced the same changes to the humus microflora as ash alone [4,5]. Without the ash this amount of Cd decreases soil respiration and changes the PLFA pattern [4].

Watering the microcosms with SAR in the field over the whole growing season slightly decreased the humus pH but did not change the interpretation of the results made above. Though there was probably a higher dissolution rate of the ash into the humus to be detected due to the SAR treatment the Cd of the wood ash was not liberated into bioavailable form and thus did not reach toxic levels. The higher dissolution of the ash induced a higher basal respiration rate. The ACd and A treatments would add 465 and 5 mg Cd m^{-2} onto the forest floor, respectively, which theoretically could leach into the environment when the ash induced pH effect stops. There are no investigations on the duration of how long the humus pH increasing wood ash effect lasts in the forest environment but it must be long, since ash induced effects on humus pH and microflora are still observed 18 years after fertilization [20]. Our results indicate that wood ash, containing Cd at levels between 1 to 30 mg Cd kg^{-1} ash, can be used in field trials to counteract soil acidification without the threat of Cd toxicity since much higher amounts of Cd spiked into the ash were not able to change any of the measured variables of this study.

The PLFA method is based on the extraction of PLFAs common in all bacteria. One of the 38 identified PLFAs is treated to be fungal. This makes the PLFA method mainly a bacterial community structure assay. It has been shown before that the humus PLFA pattern obtained from 40 environmental field plots correlated well to the bacterial PLFA pattern enriched on a nutrient rich agar media [21]. This implies that the PLFA method mainly measures changes in the dominant soil bacterial community, which can also be grown to pure culture. Using a general bacterial primer for PCR/DGGE resulted in the same resolution as the PLFA method. Therefore the use of general primers for PCR/DGGE in soil ecology pictures probably only the most dominant species of the investigated sample.

Acknowledgements

Miia Collander is thanked for assistance in the laboratory. The study was supported by the Nessling foundation.

References

[1] Staaf, H. and Olsson, B.A. (1991) Acidity in coniferous forest soils after different harvesting regimes of logging slash. Scand. J. For. Res. 6, 19–29.
[2] Steenari, B. and Lindqvist, O. (1997) Stabilisation of biofuel ashes for recycling to forest soil. Biom. Bioenerg. 13, 39–50.
[3] Bååth, E. (1989) Effects of heavy metals in soil on microbial processes and populations (a review). Water Air Soil Pollut. 47, 335–379.
[4] Fritze, H., Perkiömäki, J., Saarela, U., Katainen, R., Tikka, P., Yrjälä, K., Karp, M., Haimi, J. and Romantschuk, M. (2000) Effect of Cd-containing wood ash on the microflora of coniferous forest humus. FEMS Microbiol. Ecol. 32, 43–51.
[5] Fritze, H., Perkiömäki, J., Petänen, T., Pennanen, T., Romantschuk, M., Karp, M. and Yrjälä, K. (2001) A microcosmos study on the effects of Cd-containing wood ash on the coniferous forest humus fungal community and Cd bioavailability. J. Soils Sediments 1, 146–150.
[6] Eriksson, H.M. (1998) Short-term effects of granulated wood ash on forest soil chemistry in SW and NE Sweden. Scand. J. For. Res. Suppl. 2, 43–55.
[7] Holmberg, S.L., Lind, B.B. and Claesson, T. (2000) Chemical composition and leaching characteristics of granules made of wood ash and dolomite. Environ. Geol. 40, 1–10.
[8] Alewell, C., Manderscheid, B., Meesenburg, H. and Bittersohl, J. (2000) Is acidification still an ecological threat? Nature 407, 856–857.
[9] Pietikäinen, J. and Fritze, H. (1995) Clear-cutting and prescribed burning in coniferous forest: Comparison of effects on soil fungal and total microbial biomass, respiration activity and nitrification. Soil Biol. Biochem. 27, 101–109.
[10] Frostegård, Å., Bååth, E. and Tunlid, A. (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25, 723–730.
[11] Pennanen, T., Liski, J., Bååth, E., Kitunen, V., Uotila, J., Westman, C-J. and Fritze, H. (1999) Structure of the microbial communities in coniferous forest soils in relation to site fertility and stand development stage. Microb. Ecol. 38, 168–179.
[12] Frostegård, Å. and Bååth, E. (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–65.
[13] Heuer, H., Kresek, M., Baker, P., Smalla, K. and Wellington, E.M.H. (1997) Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl. Environ. Microbiol. 63, 3233–3241.
[14] Vainio, E.J. and Hantula, J. (2000) Direct analysis of wood-inhabiting fungi using d enaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycol. Res. 104, 927–936.
[15] Pennanen, T., Paavolainen, L. and Hantula, J. (2001) Rapid PCR-based method for the direct analysis of fungal communities in complex environmental samples. Soil Biol. Biochem. 33, 697–699.
[16] Young, F.E., Smith, C. and Reilly, B.E. (1969) Chromosomal location of genes regulating resistance to bacteriophage in *Bacillus subtilis*. J. Bacteriol. 98, 1087–1097.

[17] Tauriainen, S., Karp, M., Chang, W. and Virta, M. (1998) Luminescent bacterial sensor for cadmium and lead. Biosens. Bioelectron. 13, 931–938.
[18] Bååth, E. and Arnebrant, K. (1994) Growth rate and response of bacterial communities to pH in limed and ash treated forest soils. Soil Biol. Biochem. 26, 995–1001.
[19] Bååth, E., Frostegård, Å., Pennanen, T. and Fritze, H. (1995) Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soil. Soil Biol. Biochem. 27, 229–240.
[20] Perkiömäki, J. and Fritze, H. (2002) Short and long-term effects of wood ash on the boreal forest humus microbial community. Soil Biol. Biochem. 34, 1343–1353.
[21] Pennanen, T., Fritze, H., Vanhala, P., Kiikkilä, O., Neuvonen, S. and Bååth, E. (1998) Structure of a microbial community in soil after prolonged addition of low levels of simulated acid rain. Appl. Environ. Microbiol. 64, 2173–2180.