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Wood ash use in coniferous forests

a soil microbiological study into the potential risk of cadmium release

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Contents

ABSTRACT

Wood ash use in coniferous forests: a soil microbiological study into the potential risk of cadmium release

Wood ash fertilization could benefit the growth of forest vegetation and trees through increasing soil pH and mineral nutrient content. The use of wood ash in forestry has been questioned because the potential risk associated with its cadmium (Cd) content (1-30 mg kg^{-1}). In agriculture, wood ash is only allowed for use as fertilizer when its Cd content is below 3 mg kg^{-1}. This restriction has not been applied to forest soils and there is a lack of knowledge about the potential harmful effects of the Cd in wood ash on forest ecosystems. The main objective of this thesis was to test if the Cd in wood ash has the potential to affect the humus layer microflora of coniferous upland forests. This objective was tested both in laboratory and field experiments with ash and ash spiked with Cd (400 or 1000 mg Cd kg^{-1} as CdO or $CdCl_2$). In one study the dissolution of ash was accelerated by irrigating it with simulated acid rain (SAR).The form of the ash (loose or hardened) and dosage (3, 5 or 9 t ha^{-1}) also were investigated. In addition, the long-term (18-20 years) effects of wood ash on forest soil microbial community and decomposition rate (weight loss) of needle litter and thereby on tree growth were assessed. Also the potential of wood ash Cd to enter the human food chain were studied in the field. Finally, the potential use of wood ash as a remediation agent of heavy metal polluted soil was studied.

Wood ash increased humus layer pH and microbial activities (respiration or thymidine incorporation rates) and changed its microfloral community structure (Biolog®, PLFA, 16S or 18S rDNA PCR-DGGE) in all short-term and long-term laboratory and field experiments. Spiking ash with Cd induced no further changes in the above-mentioned variables as ash alone. The Cd added with wood ash did not become bioavailable as detected with a bacterial biosensor *Bacillus subtilis* BR151(pTOO24). The form and level of Cd added in the ash had no further effect on the microbiological variables studied. Irrigation of ash with SAR did not increase the amount of bioavailable Cd, although the dissolution rate of the ash was increased.

The results showed that, irrespective of the forest site fertility, ash fertilization induced similar chemical and microbiological responses in the humus layer. The changes were related to the dose and form of ash applied. The higher fertilization rate had stronger effects and applying loose wood ash at the same fertilization rate as hardened wood ash induced comparatively more changes, due to faster dissolution. Wood ash fertilization increased the decomposition rate of needle litter 19-20 years but not 1-4 years after treatment. On the poorer

forest site the enhancing effect of ash fertilization on needle mass loss and on Scots pine growth was more pronounced than on more fertile site.

The concentration of Cd in soil water and in *Vaccinium uliginosum* and *V. vitis-idaea* berries, and the amount of bioavailable Cd in the humus layer were not increased by the ash or Cd-spiked ash treatments in the 4 year field study. The only increase in Cd concentrations, significantly higher concentrations in the mushroom *Lactarius rufus* and a slight increase in the berries of *Empetrum nigrum* (first year only), were associated with the Cd-spiked ash treatment.

Humus layer that had been exposed to moderate amounts of continuous acid and metal (copper and nickel) deposition for nine growing seasons was used in a laboratory remediation experiment. Both acid and metal treatments changed the structure of the microbial community. Acid application decreased humus layer pH and base saturation and increased the amounts of both extractable and bioavailable Cu measured with a bacterial biosensor *Pseudomonas fluorescens* DF57-Cu15. Metal application increased the concentration of humus layer extractable Ni and changed the fungal community structure. When this humus was irrigated with water the above-mentioned treatment effects were still seen except for the acid and metal effects on microbial and fungal community structures. After treatment with wood ash, none of the acid or metal effects could be detected.

In conclusion, the Cd in wood ash did not become bioavailable and harmful to forest soil microbes or leach through the humus layer, even when treated with simulated acid rain. Neither did the concentration of Cd in the studied mushroom and berries increase with the unspiked "normal" wood ash treatment. It is thus safe to use wood ash as a vitality fertilizer in upland forests. Nevertheless it would be prudent not to fertilize the same sites with wood ash more than once during a tree stand generation. The effect of wood ash (3 t ha^{-1}) on upland forest soil microbes lasts for at least 20 years, and probably longer if higher doses and/or hardened ash are applied. In addition, wood ash can be used to remediate sites with acidified and metal polluted humus layers.

Keywords: biosensor, cadmium bioavailability, copper bioavailability, DGGE, *Empetrum nigrum,* hardened ash, *Lactarius rufus*, litter decomposition, loose ash, metal pollution, microbial activity, microbial community structure, PCR, *Pinus sylvestris*, PLFA, remediation, stem volume, thymidine incorporation, *Vaccinium uliginosum, Vaccinium vitis-idaea*

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I 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 Microbiology Ecology 32: 43-51.

II 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 humus fungal community and the Cd bioavailability. Journal of Soils & Sediments 1: 146-150.

III Perkiömäki J., Kiikkilä O., Moilanen M., Issakainen J., Tervahauta A. and Fritze H. 2003. Cadmium-containing wood ash in a pine forest: effects on humus microflora and cadmium concentrations in mushrooms, berries and needles. Canadian Journal of Forest Research 33: 2443-2451.

IV Perkiömäki J. and Fritze H. 2002. Short and long-term effects of wood ash on the boreal forest humus microbial community. Soil Biology & Biochemistry 34: 1343-1353.

V Perkiömäki J., Levula T. and Fritze H. 2004. A reciprocal decomposition experiment of Scots pine needles 19 yr after wood ash fertilization. Soil Biology & Biochemistry 36: 731-734.

VI Perkiömäki J. and Fritze H. 2003. Does simulated acid rain increase the leaching of cadmium from wood ash to toxic levels to coniferous forest humus microbes? FEMS Microbiology Ecology 44: 27-33.

VII Perkiömäki J., Tom-Petersen A., Nybroe O. and Fritze H. 2003. Boreal forest microbial community after long-term field exposure to acid and metal pollution and its potential remediation by using wood ash. Soil Biology & Biochemistry 35: 1517-1526.

Jonna Perkiömäki performed part of the experimental work, and calculation and interpretation of the results for papers I and II. In the papers III, IV, V, VI and VII she performed most of the experimental work, and calculation and interpretation of the results. Andreas Tom-Petersen performed the measurements of bioavailable Cu in paper VII. Jonna Perkiömäki wrote the papers III, IV, V and VII, and she has participated in the preparation of the manuscripts of papers I and II. Jonna Perkiömäki wrote the paper VI together with her supervisor Hannu Fritze.

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1 INTRODUCTION

1.1 Wood ash production and quality

Today about 15% of the primary energy production in Finland is from wood (Pingoud and Lehtilä 1997). Wood combustion produces ca. 250 000-300 000 t ash annually (Hytönen and Nurmi 1997). The aim of the Finnish government is that 25% of the primary energy production will be from wood and peat by the year 2005 (Hakkila and Fredriksson 1996). Thus, the amount of wood ash produced will increase in the future. The use of wood for energy production has a number of advantages over other sources - it is renewable, domestic and local, creates work and promotes silviculture (Hakkila and Fredriksson 1996). The most important justification for the use of wood bioenergy, however, is that it could significantly reduce the use of fossil fuels and their carbon dioxide emissions (Pingoud and Lehtilä 1997). The ash that is produced is often stored in waste dumps but could be recycled back to the forest ecosystem. Wood ash contains a considerable amount of mineral nutrients which could benefit the growth of forest ground vegetation and trees (Silfverberg 1996b). In order to preserve the vitality and productivity of soil the nutrients removed from a site by tree harvesting need to be returned and wood ash fertilization is one way to do this.

During combustion most of the inorganic nutrients and trace elements in wood are retained in the ash. Many factors affect the quality of wood ash, including: tree species, fraction of tree burnt, degree of processing of the tree before combustion, burning temperature and type of filters used in the incineration system, and proportion of bottom and fly ash in the end product. Hardwood ash contains significantly more phosphorus and potassium than softwood ash, which contains more calcium (Hakkila and Kalaja 1983). Bark ash has higher contents of calcium and unburnt charcoal and lower amounts of potassium, phosphorus and magnesium than stem wood ash (Hakkila 1986). The ashes of pulp and paper have lower amounts of nutrients than ashes from unprocessed wood (Demeyer et al. 2001). Residual charcoal in the ash reflects inefficient combustion which results in lower acid neutralization capacity when applied to the soil (Khanna et al. 1994). Fly ash has more charcoal, but less insoluble sand than bottom ash (Hakkila and Kalaja 1983). Dynamic separators recover 50–90%, and electric filters 90–99% of the fly ash (Hakkila and Kalaja 1983, Hakkila 1986) and the more effective the filtration of the combustion gases the higher is the proportion of heavy metals in the fly ash (Hakkila 1986, for information about wood ash Cd see chapter 1.3). The alkanity (Demeyer et al. 2001) and leachability of some heavy metals (Ramesh and Koziñski 2001, Zhang et al. 2001) of ash decreases with increasing com-

bustion temperature. Elemental concentrations in wood ash therefore show great variation.

The most abundant element in Finnish wood ash on a mass concentration basis is calcium followed by potassium and magnesium (Table 1)**.** There is more variation in the concentrations of other elements, but usually wood ash contains more phosphorus, iron and manganese than zinc, copper and boron. The amounts of sulphur and aluminum can be relatively high when the ash originates from paper and pulp industry. The amount of sulphur in wood ash is seldom measured/reported, however, probably because it is assumed that most of the sulphur is lost from the ash to the air with combustion gases (Hakkila and Fredriksson 1996, Silfverberg 1996a, Eriksson 1998a). However, according to Obernberger (1998), 40-90% of the total S in biofuel remains in the ash and the rest is emitted as SO_2 and SO_3 with the flue gas. The amounts of carbon and nitrogen are negligible in wood ash because they are generally oxidized and transformed to gaseous constituents during combustion (Demeyer et al. 2001). Khanna et al. (1994) grouped the elements in wood ash to those that rapidly dissolve (>50% of total content) (K, B, and S), those that dissolution increases with increasing dilution of ash with water (Ca, Mg, Si, Fe, and Al) and those that are quite insoluble (P).

During the combustion of wood, organic compounds are mineralized and the base cations are transformed to their oxides which then slowly hydrate and subsequently carbonate under atmospheric conditions (Demeyer et al. 2001). Oxides and hydroxides are the more reactive while carbonates the more slowly reactive fractions of wood ash (Steenari and Lindqvist 1997). During spreading fluffy loose wood ash its reactive fractions are detrimental to human health (Juntunen 1982), and they can erode parts of the spreading machines (Hakkila and Kalaja 1983), cause salt effects in soils and burn damage to plant tissues (see chapters 1.2.1 and 1.2.2 below). In order to reduce these harmful effects

Table 1. Element concentrations in Finnish wood ashes.

Nutrient	Content in ash (kg t^{-1}) (means± SD; n = 130-199)
Ca	226 (81)
K	47.8 (33.8)
Mg	32.7 (15.5)
Fe	22.9 (34.6)
P	15.5 (9.7)
Mn	14.9 (8.0)
Zn	1.66 (1.65)
B	0.27 (0.25)

Data from Silfverberg 1996a.

of wood ashes one can stabilize them before spreading in forest by self-hardening (compacting in a pile), pelletizing (pressing to form pellets) or granulating (granulating in a drum or a disc) (Holmberg and Claesson 2001). In all these stabilization techniques ash is mixed first with water and in pelletation or granulation a binder (e.g. dolomite) is used (Holmberg and Claesson 2001). Self-hardening is the cheapest technique to stabilize wood ash. In hardening process the reactivity of wood ash is reduced by hydroxide formation and carbonation of hydroxides ($Ca(OH)_2 \rightarrow CaCO_3$) (Steenari and Lindqvist 1997). Calcite ($CaCO_3$) is approximately a hundred times less soluble than calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$) (Steenari et al. 1999). Carbonation also reduces the alkanity of the ash (Steenari and Lindqvist 1997).

1.2 Wood ash effects in the coniferous forest ecosystem

The following chapters summarize the knowledge about the effects of wood ash fertilization in the coniferous forest ecosystem (shortly summarized in Table 2).

1.2.1 Soil and water chemistry

The most commonly reported changes on humus layer chemistry following wood ash fertilization are a decrease in extractable Al concentrations, a rise in pH and in the concentrations of base cations (Khanna et al. 1994, Bramryd and Fransman 1995, Kahl et al. 1996, Levula et al. 2000, Saarsalmi et al. 2001, Ludwig et al. 2002).

For N and P the effects of wood ash fertilization are not so clear. Wood ash application was found to increase the amount of total N and extractable P in the humus layer only in one of the four studied forests 16 years after application (Saarsalmi et al. 2001). Levula et al. (2000) and Tamminen (1998) also observed an increase in extractable P contents after wood ash treatment. Fritze et al. (1995) and Arvidsson and Lundkvist (2003) did not observe an ash effect on the amount of humus layer N contents and Kahl et al. (1996) and Fransson et al. (1999) did not observe an increase for extractable P contents. The amount of total N in the humus layer has sometimes been shown to decrease after wood ash treatment (Fritze et al. 1994b, Haimi et al. 2000).

Because nutrient concentrations in the groundwater of peatlands have been shown to increase after wood ash treatment (Nilsson and Lundin 1996, Piirainen 2001, Moilanen et al. 2002), there has been concern about a possible threat that applying wood ash could cause to watercourses. The following summarizes the knowledge about the effects of wood ash fertilization on min-

eral (upland) soil water quality. In the studies by Fransman and Nihlgård (1995) and Ring et al. (1999), wood ash application did not change pH or the concentrations of Ca, SO^{2-}_4, NO^-_3 and Al in soil water. Neither did Fransson et al. (1999) find increase in soil water pH or concentration of P nor Arvidsson (2001) recorded an increase in soil water pH or the concentrations of NO^-_3 and Al. However, there are also studies where a rise in soil water pH and the concentrations of these ions has been observed due to wood ash application. In the study by Kahl et al. (1996) there was a rise in pH and Ca, SO^{2-}_4 and NO^-_3 concentrations after wood ash fertilization (24 and 38 t ash ha^{-1}). All the changes, except the rise in solution pH and SO^{2-}_4 concentrations at the application level of 38 t ash ha^{-1} were transient. In the study by Lundell et al. (2001) there was a clear increase of Ca and a slight increase in the leaching of SO^{2-}_4 and Al after wood ash addition even though the application level was quite low (4 t ha^{-1}). Arvidsson (2001) also observed increased levels of Ca in soil water after low application of wood ash (3 t ha^{-1}), but they decreased to control levels within five years after application at most of the studied sites. When Ludwig et al. (2002) applied 4.8 t $ha^{-1}$of wood ash to one forest plot (no replications), they observed an increase in the concentrations of Ca, SO^{2-}_4, NO^-_3 and Al in soil water, but these changes were absent after two years.

Fransman and Nihlgård (1995) studied the quality of runoff water from forested areas for 4 years after wood ash treatment (2.2 t ha^{-1}) and the only change they observed was an increase in the concentration of K. In the study by Tulonen et al. (2002), performed in a forest growing on mineral soil, 12% of the catchment area was treated with wood ash (6.1 t ha^{-1}) and resulted in a slight increase in runoff (annual average) and recipient lake water pH and K concentrations, but no increase in Ca, P and N concentrations occurred. Wood ash fertilization thus does not appear to lead to the eutrophication of watercourses as there is negligible leaching of N and P. Wood ash treatment has, however, been found to increase dissolved organic carbon (DOC) concentrations in some studies (Weber et al. 1985, Khanna et al. 1994, Ludwig et al. 2000, Chirenje et al. 2002). Yet Parkman and Munthe (1998) did not observe increased DOC concentrations in runoff water from a study area treated with granulated wood ash (2.2 t ha^{-1}) 4-5 years earlier. There is a lack of knowledge about the effects of wood ash on upland forest groundwater quality.

A salt-effect is sometimes observed after wood ash application (Eriksson et al. 1998), whereby soil pH initially (during the first month) decreases with increasing level of ash because of increased ionic strength and displacement of H and Al from exchange sites by other cations dissolving from the ash. Later on, when the carbonates and oxides dissolve to a higher degree than neutral salts, the ash has a more direct alkalizing effect (Eriksson et al. 1998). A temporary decrease in soil water pH has also been observed in the first year after wood ash addition (4.8 t ha^{-1}) (Ludwig et al. 2002).

In summary, the effects of wood ash fertilization on the chemical properties of upland forest soils are beneficial - soil acidity is decreased and base cation

concentrations, which are important plant nutrients, increased. Eutrophication of watercourses related to wood ash treatment has not been observed.

1.2.2 Forest floor and ground vegetation

The effect of wood ash on vegetation depends on the development stage of the forest stand (Pihlström et al. 2000), site fertility (Silfverberg and Huikari 1985, Arvidsson et al. 2002), form (Rühling 1996, Kellner and Weibull 1998) and dosage (Silfverberg and Huikari 1985, Ferm et al. 1992, Jacobson and Gustafsson 2001) of the ash applied. Although the effects of wood ash are not consistent in all studies performed in upland forests, some generalization can be made.

The coverage of herbs such as *Epilobium angustifolium (*Pihlström et al. 2000, Arvidsson et al. 2002, Olsson and Kellner 2002), *Trientalis europaea* (Gyllin and Kruuse 1996, Arvidsson et al. 2002), *Melampyrum* (Gyllin and Kruuse 1996, Pihlström et al. 2000, Jacobson and Gustafsson 2001) and *Taraxacum* (Rühling 1996, Olsson and Kellner 2002), grasses such as *Deschampsia flexuosa* (Silfverberg and Issakainen 1991, Gyllin and Kruuse 1996, Arvidsson et al. 2002) and *Luzula pilosa* (Gyllin and Kruuse 1996, Olsson and Kellner 2002), and shrubs such as *Rubus ideaus* (Rühling 1996, Arvidsson et al. 2002) increased. The increase in the coverage of *E. angustifolium* and *D. flexuosa* has been attributed to higher nitrogen availability in the soil (Arvidsson et al. 2002).

The coverage of dwarf shrubs decreased in some studies (e.g. *Vaccinium vitis-idaea* - Levula et al. 2000, Jacobson and Gustafsson 2001; *Vaccinium myrtillus* - Jacobson and Gustafsson 2001 and *Calluna vulgaris* - Jacobson and Gustafsson 2001, Arvidsson et al. 2002) while increased in other (e.g. *V. vitis-idaea* and *V. myrtillus* - Pihlström et al. 2000). The berry yields of *V. vitis-idaea* and *V. myrtillus* have also been shown to increase after wood ash fertilization (Silfverberg and Issakainen 1991), but the berry yield of *V. myrtillus* decreased in the study by Moilanen and Issakainen (2000).

Wood ash has caused salt-burn damage to bryophytes *Dicranum polysetum*, *Pleurozium schreberi* and *Hylocomium splendens* (Kellner and Weibull 1998, Jacobson and Gustafsson 2001). Despite of the burn damage on *H. splendens*, no changes in its coverage were observed (Kellner and Weibull 1998, Jacobson and Gustafsson 2001), but that of *D. polysetum* (Jacobson and Gustafsson 2001) and *P. schreberi* (Kellner and Weibull 1998) decreased. In the study by Kellner and Weibull (1998) a significant negative relationship between burn damage and the photosynthetic capacity of the bryophytes was found. One bryophyte, the calciphilic *Pohlia nutans*, benefited from wood ash fertilization (Gyllin and Kruuse 1996, Jacobson and Gustafsson 2001). Lichens *Cladonia* (Gyllin and Kruuse 1996, Pihlström et al. 2000) and *Cladina* (Gyllin and Kruuse 1996, Jacobson and Gustafsson 2001) suffer from wood ash treatment. There are also studies were no changes in the cover of bryophytes (Levula et

al. 2000, Arvidsson et al. 2002) or lichens (Kellner and Weibull 1998, Levula et al. 2000) were found.

In many long-term wood ash fertilization studies performed on peatland an increase in the coverage of herbs and grasses and colonization by nitrophiles (e.g. *E. angustifolium*) has also been observed (Lukkala 1951, Silfverberg and Huikari 1985, Silfverberg and Hotanen 1989, Ferm et al. 1992, Moilanen et al. 2002). In addition, *Sphagnum* mosses are substituted by forest mosses. These changes in vegetation could result in increased decomposition of needle litter and improvement in nutrient cycling and thereby increased tree growth (Silfverberg and Huikari 1985).

In conclusion, in upland forests the changes in the composition of the forest floor and ground vegetation after wood ash treatment were quite small (Levula et al. 2000, Jacobson and Gustafsson 2001, Arvidsson et al. 2002). In the study by Pihlström et al. (2000) the changes in the vegetation following wood ash application were mainly changes in the abundance of existing species and not in the appearance of the new species and disappearance of the old ones. However, Gyllin and Kruuse (1996) and Arvidsson et al. (2002) observed a slight increase in species richness after wood ash application. In the peatland forests the changes in the vegetation were more pronounced.

1.2.3 Trees

1.2.3.1 Growth

In the study by Silfverberg (1995) soaking (one day or week) Scots pine (*Pinus sylvestris)* seeds in a wood ash solution decreased their germination, while Rikala and Jozefek (1990) did not find an ash effect on the germination capacity of Scots pine or Norway spruce (*Picea abies*) on peat. Wood ash treatment on peat increased the number (Silfverberg 1995) and growth (Kaunisto 1987, Rikala and Jozefek 1990) of Scots pine seedlings and decreased their growth disturbances caused by nutrient imbalances (Kaunisto 1987). The higher number of surviving seedlings indicates that wood ash improves the forest regeneration.

Wood ash has also been shown to increase the growth of Scots pine on peatlands in many long-term (9-47 years) experiments (Lukkala 1951, Silfverberg and Huikari 1985, Silfverberg and Hotanen 1989, Silfverberg 1991, Ferm et al. 1992, Moilanen and Issakainen 2000, Moilanen et al. 2002, Hytönen 2003). Usually the ash effect was stronger when the used dose was higher (Silfverberg and Huikari 1985, Silfverberg and Hotanen 1989), but not always (Silfverberg and Huikari 1985). In addition, the growth increment of trees was higher on peat with higher nitrogen content (Lukkala 1951, Silfverberg and Huikari 1985, Silfverberg 1991). According to Moilanen and Issakainen (2000), the increase in the growth increment appeared sooner (2-3

years after application) on sites with higher nitrogen contents than on poorer sites (7-8 years after application).

There are few publications in Finland concerning wood ash fertilization effects on tree growth on mineral soils. A Finnish review article (Saarsalmi and Mälkönen 2001) concluded that wood ash does not increase the growth of coniferous upland forests on mineral soils. According to a Swedish review (Nohrstedt 2001), wood ash stimulated forest growth on sites with a humus C-to-N ratio below 30, while on N-poor sites the effect could be reduced growth. When Tamminen (1998) studied the effect of wood ash fertilization (3 t ha^{-1}) on the height growth of 8-15-years old Scots pines he observed an increase in growth 2-5 years after fertilization (2 replications). Sikström (1992) did not observe increased conifer tree (Scots pine and Norway spruce) growth during 5 year study period in stands fertilized with quite low doses (0.3 and 0.5 t ha^{-1}) of wood ash. Jacobson (2001) found the same result 7-11 years after fertilization but with higher doses (1, 3, 6, 9 t ha^{-1}) unless nitrogen (150 kg ha^{-1}) was also applied. However, in the study by Jacobson (2001) there was an increasing, although not significant, trend in tree growth on fertile sites treated with wood ash. Levula (1991) found that when nitrogen (180 kg N ha^{-1}) was added with wood ash (2 t ha^{-1}) the volume increment of Scots pine 9 years after ash application was ca. 73%. In the study by Saramäki and Susila (1991) the increase in Scots pine volume growth after wood ash application (5 t ha^{-1}) with nitrogen (urea 400 kg ha^{-1}) during 10 years was ca. 60%, and without nitrogen it was 17% (no replications). In another Finnish study, Moilanen and Issakainen (2000) found that wood ash treatment (3.6 and 7.2 t ha^{-1} 10 years earlier) did not increase the growth of Scots pine, except on the plot where N in addition to ash (4.5 t ha^{-1} 19 years earlier) also have been applied.

The effects of peat ash on tree growth have also been studied. However, the acid neutralization capacity (Saarela 1991) and nutrient contents (especially K) are not as high as in wood ash, but it has been considered as a phosphorus fertilizer (Hakkila 1986, Saarela 1991, Silfverberg and Issakainen 1991, Issakainen et al. 1994, Oikarinen and Pasanen 1994). Thus, more peat ash than wood ash is needed to achieve the same growth increment for Scots pines (Kaunisto 1987, Saramäki and Susila 1991, Issakainen et al. 1994, Moilanen and Issakainen 2000, Hytönen 2003).

In summary, while wood ash fertilization has been shown to generally increase the growth of coniferous trees on peatlands forests this is not the case with upland forests unless extra nitrogen is also applied.

1.2.3.2 Foliar chemistry

In tree stands growing on mineral soils P and especially N deficiency is quite common (Raitio et al. 2000). A boron deficiency also sometimes occurs. While wood ash treatment has not been shown to increase foliar N concentrations in conifer trees growing on mineral soils (Vuorinen and Kurkela 2000, Jacobson 2001), it has been shown to increase B concentrations (Moilanen and

Issakainen 2000, Jacobson 2001, Nohrstedt 2001). However, Arvidsson and Lundkvist (2002) have shown increased Norway spruce needle N concentrations after ash treatment, and attributed it to increased mineralization of soil N (see below chapter 1.2.4.1). Also needle P (Kaunisto 1987, Jacobson 2001, Arvidsson and Lundkvist 2002) and K (Kaunisto 1987, Moilanen and Issakainen 2000, Jacobson 2001, Arvidsson and Lundkvist 2002) concentrations have been shown to increase after wood ash treatment. In the studies by Vuorinen and Kurkela (2000) and Ludwig et al. (2002), wood ash fertilization was found to not affect the nutrient concentrations of Scots pine needles.

In peatland forests P and K (Silfverberg and Huikari 1985, Silfverberg and Hotanen 1989, Moilanen et al. 2002), and B (Kaunisto and Paavilainen 1988) deficiency may occur. Studies in peatlands have found that wood ash treatment sometimes increases nutrient concentrations in Scots pine needles and sometimes has no effect. These effects were not consistent for all nutrients, but generally needle concentrations of K (Silfverberg and Hotanen 1989, Ferm et al. 1992, Silfverberg et al. 1994, Moilanen and Issakainen 2000, Moilanen et al. 2002, Hytönen 2003) and B (Silfverberg and Issakainen 1987, Ferm et al. 1992, Moilanen and Issakainen 2000, Hytönen 2003) have been found to increase after wood ash fertilization. Increases in needle P concentrations have also been observed (Silfverberg and Hotanen 1989, Silfverberg et al. 1994, Moilanen and Issakainen 2000, Moilanen et al. 2002). These increases in needle P, K and B concentrations have been found to be long-term; lasting for 51, 51 and 26 years, respectively (Moilanen and Issakainen 2000). Long-term increases in needle N concentrations on peatlands after wood ash treatment have also been recorded (Silfverberg and Hotanen 1989). Wood ash fertilization has no effect on needle nutrient concentrations in stands with originally good nutrient status (Silfverberg and Huikari 1985, Silfverberg 1991).

In summary, wood ash fertilization has often been shown to increase concentrations of P, K and B in Scots pine needles, both on uplands and peatlands.

1.2.3.3 Diseases

In upland forests wood ash treatment has been shown to have little or no effect on the occurrence of tree diseases. Wood ash fertilization did not affect the *Lophodermella sulcigena* infection of Scots pine needles, but nitrogen fertilization increased it (Vuorinen and Kurkela 2000). The infection frequency of *Heterobasidion annosum*, the causative agent of root rot in Scots pine roots, was not higher and average mycelia growth was lower on plots treated with wood ash (Piri 2000). In the study by Tamminen (1998), wood ash had no significant effect on the occurrence of Scots pine flatbug infection (*Aradus cinnamomeus*) although a slight decreasing trend was seen. Also in peatland forests wood ash fertilization has been shown to increase the proportion of healthy trees (Kaunisto 1987, Ferm et al. 1992, Hytönen 2003).

In conclusion for the chapter 1.2.3 (effects on trees)., wood ash can be successfully be used to remediate soils by reducing soil acidity and the depletion of nutrients rather than a fertilizer to increase the growth of coniferous trees directly.

1.2.4 Decomposers

1.2.4.1 Microbes

Microbial biomass has commonly been measured using substrate-induced respiration (SIR), fumigation-extraction (FE) and the ATP content of the soil. The PLFA method has also become popular. In this method the phospholipid fatty acid content of the microbial cell membranes is measured. PLFA analysis can also be used to reveal changes in the structure of the microbial community and signature fatty acids can be used to indicate of certain groups of microorganisms (see below).

In general wood ash treatment has been shown to have no effect on the microbial biomass of the boreal forest humus layer in a number of field studies. Thus, no change in SIR (Priha and Smolander 1994) or FE (Fritze et al. 1994b, Priha and Smolander 1994), or ATP content of the soil (Bååth and Arnebrant 1994) was reported. However, Bååth and Arnebrant (1994) observed an increase in SIR after wood ash fertilization. Bååth and Arnebrant (1994) also observed an increase in the amount of culturable bacteria, but the acridine-orange stained direct counts (AODC) did not change. In contrast, Bååth et al. (1995) observed a decrease in total amounts of phospholipid fatty acids (PLFAs) after wood ash fertilization, but this decrease was only significant at the highest application level (5 t ha $^{-1}$). In addition to application level of wood ash, also the exposure time to ash has been shown to affect on the strength of microbial biomass response. Frostegård et al. (1993a) did not find an ash effect on the total amounts of PLFAs 5 years after the treatment at the same site where Bååth et al. (1995) found an ash effect two years after treatment. When bacterial and fungal biomasses in the humus layer after wood ash treatment have been measured separately with PLFA analysis Frostegård et al. (1993a) found no change in either while Bååth et al. (1995) found them both to decrease. When fungal biomass was measured as ergosterol content, no change following ash fertilization was observed by Fritze et al. (1994b).

Although the microbial biomass may not change after ash treatment its activity may. In most of the wood ash studies performed in boreal forests, microbial activity in humus layer, measured as either mineralization activity (basal respiration rate measured as CO_2 production; Bååth and Arnebrant 1994, Fritze et al. 1994b, Fritze et al. 1995) or growth rate (thymidine incorporation rate; Bååth and Arnebrant 1994, Bååth et al. 1995, Hagerberg and Wallander 2002), increased. However, in the study by Priha and Smolander (1994), wood ash treatment (2.5 t ha^{-1}) did not have an effect on respiration rates one year after application.

Raison and McGarity (1980) observed that ash addition to soil (sandy podzolic soil) increased the respiration rate (measured as CO_2 production), compared to a sterilized control soil. This indicates that ash has a direct effect on microbial activity. In addition, they also showed that the elevation of soil pH was the major factor, which led to the increased respiration rate as neutralized ash did not give a respiration response. An increased respiration rate is usually taken to indicate increased mineralization and nutrient cycling, but it may also indicate a stress response by soil microbes due to increase in soil pH. Bacterial communities in ash treated soils have, however, been shown to be able to adapt to increased humus layer pH (Bååth and Arnebrant 1994, Bååth et al. 1995).

The mineralization activity of microbes can also be measured as the mass loss of needle litter. In the study by Smolander et al. (1996), wood ash fertilization increased the mass loss of Scots pine needle litter.

Wood ash fertilization has also been shown to increase net nitrification (Martikainen 1984) and the numbers of autotrophic nitrifiers (NH_4 and NO_2 oxidizers) (Martikainen 1985a) in humus layer samples one and two years after application. Five years after wood ash application nitrification rates were no longer elevated above controls, unless ammonium had also been added with ash (Martikainen 1985b). Neither did Fritze et al. (1994b) observe increased nitrification 2 years after wood ash application. Increased nitrification may lead to nitrate leaching from the soil (see chapter 1.2.1).

The effects of wood ash fertilization on soil microbes have also been studied in peatland forests. Microbial activity has been measured as respiration rate (Silvola et al. 1985, Ferm et al. 1992), decomposition rate of needles (Silfverberg and Huikari 1985, Silfverberg and Hotanen 1989) and cellulose (Karsisto 1979, Weber et al. 1985, Moilanen et al. 2002), and all have increased following ash application. Also the abundance of many bacterial groups were increased by wood ash. Karsisto (1979) observed an increase in the total number of bacteria, starch decomposing, glucose fermenting and lipolytic bacteria 53 years after wood ash treatment and Weber et al. (1985) in the numbers of aerobic, amylolytic, denitrifying and clostridial bacteria 2 years after wood ash treatment. In both these studies the numbers of proteolytic, ammonifying and ureolytic bacteria and the net mineralization of N increased. In the study by Huikari (1953) wood ash application increased the amounts of aerobic bacteria and yeasts in the surface layer of peat, but decreased the amounts of moulds.

PLFA analysis, which has been shown to be a sensitive measure of environmental changes (Pennanen et al. 1996, Pennanen et al. 1998, Bååth et al. 1998), has been used to study the effects of wood ash on forest soil microbial community structure. In the study by Frostegård et al. (1993a), wood ash treatment induced a change in humus layer PLFA pattern but not in microbial biomass five years after treatment, which indicated that some species suffered from wood ash treatment while others benefited and compensated for the loss in biomass caused by more sensitive species. Also Bååth et al. (1995) observed

a change in humus layer microbial community structure two years after wood ash fertilization. In both these studies the changes in microbial community structures correlated with humus layer pH. Fritze et al. (1994a) found that humus layer quality analyzed with near-infrared spectroscopy (NIR) was not significantly different between wood ash treated and control samples. However, when Bååth et al. (1995) performed an infrared spectroscopy (IR) analysis of humus layer samples they did find difference between wood ash treated and control samples. Bååth et al. (1995) concluded that the changes in humus layer microbial community structure were related to humus layer quantity (availability of organic matter), quality and pH. The availability of organic matter may be related to increased amounts of dissolved organic carbon (DOC) that has sometimes been observed after wood ash application (see chapter 1.2.1). In addition to increased amounts of DOC, the amount of dissolved organic nitrogen (DON) has been shown to increase after wood ash addition (Ludwig et al. 2000). N is an important macro nutrient for microbes, although microbial growth in soils is generally C limited (Aldén et al. 2001).

A deeper sight into the changes of microbial community with PLFA analysis may be achieved by using signature fatty acids specific for certain groups of microorganisms. For example, there are signature fatty acids for fungi (18:2ω6; Federle 1986, Frostegård and Bååth 1996), arbuscular mycorrhiza (AM) fungi e.g. *Glomus* species (16:1ω5; Graham et al. 1995, Olsson et al. 1995, Larsen et al. 1998) and actinomycetes (10Me16:0, 10Me17:0, and 10Me18:0; Kroppenstedt 1985). In their AM fungal studies performed in the laboratory, Larsen et al. (1998) and Olsson et al. (1998) used 18:2ω6 as an indicator of saprophytic fungi, while Olsson and Wallander (1998; laboratory study) and Hagerberg and Wallander (2002; field study) used it as an indicator of ectomycorrhizal (EM) fungi. Since mycorrhizal hyphae form a substantial part of fungal biomass in soil (Finlay and Söderström 1989), the amount of 18:2ω6 in field soil sample probably indicates the amount of EM fungi. EM are mainly associated with woody plants, including the genera *Pinus* and *Picea*, while AM are associated with herbs and grasses. It is noted that the coverage of herbs and grasses often increases after wood ash fertilization (see chapter 1.2.2). In the studies by Frostegård et al. (1993a) and Bååth et al. (1995), the amount of PLFAs 16:1ω5 and 10Me18:0 increased after ash application. Frostegård (1993a) did not observe change in the amount of PLFA 18:2ω6, but Bååth et al. (1995) observed a significant decrease at the highest dose of wood ash (5 t ha^{-1}).

EM fungi play an important role in the nutrition of trees because they can transfer organic and inorganic nutrients from soil to the colonized tree roots. Nitrogen deficiency is common in boreal forest ecosystems because only a small fraction of total soil N is available to plants. EM fungi infection have been shown to improve the nitrogen acquisition of trees through providing access to organic N (France and Reid 1983, Finlay and Söderström 1989, Chalot and Brun 1998) and increase the uptake of ammonium (NH_4^+) (France and Reid 1983, Rygiewicz et al. 1984a, Wallander et al. 1999) and nitrate

(NO_3^-) (France and Reid 1983, Rygiewicz et al. 1984b). The response of EM fungi to wood ash fertilized soil is thus important, as wood ash contains negligible amounts of N itself.

As EM fungi have been shown to colonize (Mahmood et al. 2002) and solubilize wood ash (Mahmood et al. 2001, 2003) they may affect the acquisition of N and other nutrients in wood ash treated forests. Phosphorous in wood ash is primarily bound in apatite and other compounds of low solubilities (Steenari and Lindqkvist 1997, Nieminen 2003). EM fungi may be expected to play an important role in mobilizing the P in wood ash (Mahmood et al. 2001, 2003). In wood ash treated soil microbial biomass (including mycorrhizal extramatrical hyphae) has been found to store P and it could be released for use of trees (Clarholm 1998). EM fungi have also been shown to release Ca from the wood ash and transport it to the roots of Norway spruce (Hagerberg 2003, Wallander et al. 2003, Mahmood et al. 2003).

A third wood ash nutirient possibly affected by EM fungi is K, which significant fraction is rapidly released from wood ash (Eriksson 1998b, Steenari et al. 1998, Steenari et al. 1999, Holmberg et al. 2000, Hagerberg and Wallander 2002, Nieminen 2003). As some EM fungi have been shown to have high capacity to accumulate K (Kottke et al. 1998, Wallander et al. 2003), they could play an important part in hindering the leaching of K.

Studies have shown that wood ash treatment does not have detrimental effects on EM fungi. Indeed, wood ash has been found to stimulate EM mycelium production (e.g. Ohtonen and Tuohenmaa 1999, Mahmood et al. 2001, Hagerberg and Wallander 2002). Wood ash treatment has been shown to have no (Erland and Söderström 1991) or only minor effects (Mahmood 2000) on EM community structure in coniferous tree forests. However, some negative effects on EM fungi have been observed after wood ash treatment. Rühling (1996) found that while the occurrence of fruiting bodies of saprophytic fungi (e.g. *Clitocybe*, *Lycoperdon*) increased those of mycorrhiza-forming species (*Russula, Boletus* s.l.) decreased after ash application. Erland and Söderström (1991) observed a decrease in the number of mycorrhizal root tips per meter root in Scots pine seedlings 4 months after wood ash fertilization. But Mahmood et al. (2002) did not observe a change in the number of mycorrhizal root tips of Norway spruce seedlings 7 years after ash application.

In summary, the effects of wood ash on forest soil microbes are stimulative. Mineralization activity is increased leading to improved nutrient cycling. Because wood ash does not contain nitrogen, microbes and especially mycorrhizal fungi play an important role in improving the acquisition of soil nitrogen for transfer to trees.

1.2.4.2 Fauna

Although microbes play a major role in decomposition processes in forest soils also soil fauna either directly or indirectly increase organic matter decomposition and nutrient mineralization (Faber and Verhoef 1991, Heneghan and

Bolger 1998, Edsberg 2000), and subsequently coniferous tree seedling growth (Jentschke et al. 1995, Setälä 1995). The dominant soil animal groups in coniferous forest soils in terms of biomass are enchytraeids and earthworms, followed by mites, spiders, beetles, nematodes, collembolas, protozoans, rotifers and dipterous larvae (Huhta et al. 1998). Enchytraeids and earthworms influence more the soil processes in boreal forest soil than microarthropods (mites; Oribatida, Mesostigmata, Prostigmata, and collembolas) and nematodes (Huhta et al. 1998).

The effect of wood ash fertilization on the abundance of enchytraeids has varied. Lundkvist (1998) and Liiri et al. (2002b) did not find a reaction to wood ash treatment while Huhta et al. (1986) observed a decrease in the biomass of enchytraeids following forest wood ash application and Haimi et al. (2000) found the numbers of *Cognettia sphagnetorum*, which was the only enchytraeid worm species, decreased. *C. sphagnetorum* is the dominant enchytraeid worm species and forms the main part of the soil animal biomass in northern European coniferous forests (see review Huhta et al. 1998). In addition *C. sphagnetorum* may be a keystone species in these soils (Huhta et al. 1998). Lundkvist (1998; ash with NH_4 addition) found an increased abundance of earthworms in wood ash treated soil while Huhta et al. (1986) found no earthworms in control soils and only a few in ash treated soil. Both Huhta et al. (1986) and Liiri et al. (2002b) found an increase in the number of nematodes, especially bacterial feeders related to wood ash treatment.

Huhta et al. (1986) and Haimi et al. (2000) observed a decrease in the total abundance of microarthropods following forest wood ash fertilization, but this was not found by Liiri et al. (2002a, b), probably because of the lower increase in humus layer pH. The effect of wood ash fertilization on the number of collembolas has been neutral (Haimi et al. 2000), positive (Huhta et al. 1986) or negative (Liiri et al. 2002a). The reaction of mites to wood ash fertilization has also been varied with stimulation (Huhta et al. 1986; Prostigmata), inhibition (Huhta et al. 1986; Mesostigmata, Oribatida, Haimi et al. 2000; Oribatida) or no reaction (Haimi et al. 2000; Mesostigmata, Liiri et al. 2002a; Mesostigmata, Oribatida) in their abundance being reported.

All the above-mentioned changes observed by Haimi et al. (2000) were caused with an ash application dose of 5 t ha^{-1}, but not with 1 t ha^{-1}. In addition to the changes in the abundance of some soil fauna groups, changes in the vertical distribution have also been observed after wood ash doses of 5 (Haimi et al. 2000), 7 (Huhta et al. 1986) and 8 t ha^{-1} (Lundkvist 1998), but not with lower doses. All these studies were performed in the field. In the study by Huhta et al. (1986) wood ash effect on soil fauna was stronger in the laboratory than in the field, but the results were generally in good accordance. Neither did Liiri et al. (2002a) find a discrepancy between the wood ash effects on soil fauna in their field and laboratory experiments.

In summary, although some soil fauna groups and species suffer from wood ash treatment, soil fauna in general show little response to wood ash fertilization (Haimi et al. 2000, Liiri 2001). Even in the study by Huhta et al. (1986),

Table 2. A summary of general effects of wood ash fertilization on coniferous, upland forests from the publications cited in this thesis

Compartment of ecosystem	Effect of wood ash fertilization
Trees	- little effect on growth - increase in needle P, K and B content - little effect on disease occurrence
Forest floor and ground vegetation	- a slight increase in abundance of herbs and grasses and decrease of lichens and bryopytes
Humus layer	- rise in pH and base cation content - increase in carbon mineralization - change in microbial community structure - little response on faunal community - stimulation of EM mycelium production - nutrient solubilization from ash by EM fungi which then accumulate nutrients or transport them to tree roots
Soil water	- little effect on soil water quality
Runoff and lake water	- a slight increase in pH and K content
Groundwater	- lack of knowledge

where the effect of ash on certain soil fauna was negative, the biomass of soil fauna was much higher in ash treated soil than in controls if earthworms, which have a strong influence on soil processes, were included in the calculation. This was attributed to the rise in soil pH brought about by the wood ash.

1.3 Wood ash harmful substances

Although wood ash has many beneficial effects when spread in forest ecosystems, as stated in chapters above, it does contain toxic substances, which may be harmful. These toxic substances include organic compounds (polyaromatic hydrocarbons, chlorobenzenes, and chlorophenols), ^{137}Cs activity and heavy metals (Cu, Zn, Mn, Pb, Cd, Cr, Hg, and Ni).

The levels of polyaromatic hydrocarbons (PAH) in wood ash granules were below 0.16 mg kg^{-1} in stored granules and 6.7 mg kg^{-1} in fresh granules in the study by Holmberg et al. (2000). Ash granules are sometimes dried in order to increase their hardening rate and this drying process affects the concentration of PAHs in granules. When dried with flue gas at a heating plant, PAH concentrations were 0.8 mg kg^{-1} compared to 1.4 mg kg^{-1} when dried under hot air (Holmberg et al. 2003). However, PAH concentrations in wood ash can be high. In the study by Bundt et al. (2001), wood ash total PAH concentration was 16.8 mg kg^{-1} and one year after its application (8 t ha^{-1}) in the forest, con-

centrations in the organic horizon were up to six fold higher than those in controls. According to Danish legislation, the content of PAH in wood ash fertilizer should not exceed 3 mg kg^{-1} d.m. (Møller and Ingerslev 2001). Currently, there are no limits set in Finnish legislation.

The Chernobyl nuclear accident in 1986 resulted in the deposition of radioactivity to forest ecosystems in Finland and Sweden. This has led to increased radioactivity of wood ash. The Swedish Radiation Protection Institute recommends that no wood ash with radioactivity exceeding 5 kBq kg^{-1} should be applied to forests (Högbom and Nohrstedt 2001). In the study by Högbom and Nohrstedt (2001) the application of wood ash (3 t ha^{-1}) contaminated with ^{137}Cs (0 - 4.8 kBq kg^{-1}) did not change or even decreased the ^{137}Cs activity within forest soil and vegetation at one studied site. One explanation for the decrease in radioactivity given was the antagonistic effect of K occurring in wood ash. In another Swedish study, part (11 - 24%) of the ^{137}Cs (1.9 - 2.1 kBq kg^{-1}) in ash granules (4 t ha^{-1}) leached into the soil and transferred from there to trees during 5 year period (Ravila and Holm 1996). However, no ^{137}Cs activity was observed in the soil water at depths of 20 and 50 cm. In Finland, Levula et al. (2000) studied the effect of wood ash (5 t ha^{-1}) having ^{137}Cs concentrations of 5 - 10 kBq kg^{-1}, and they found that wood ash application decreased the ^{137}Cs activity of lingonberries (*Vaccinium vitis-idaea*).

Because of its severe toxicity and relatively high mobility, cadmium (Cd) is considered to be the most harmful heavy metal in the wood ash. When Wang et al. (2001) studied the partitioning of Cd during combustion of municipal solid waste they observed that about 80% of Cd was partitioned in flue gas, about 20% in fly ash and none in bottom ash. Because these partitioning measurements of Cd are from incineration of municipal waste they are not strictly comparable to incineration of wood. Cd content of wood ash tends to increase with decreasing particle size (Zhan et al. 1996, Obernberger et al. 1997) and thus Cd concentrates in fine fly ash fraction (Narodoslawsky and Obernberger 1996). Fly ash forms the major part of the ash produced in modern incineration systems (circulating fluidized bed boilers; Eriksson et al. 1998) used by Finnish forest companies. In addition, today the amounts of heavy metals in wood ash have increased due to better filtering in the incineration systems. The Cd concentration of wood ash (varying between 1 - 30 mg kg^{-1} ash; Steenari and Lindqvist 1997) is generally higher than is allowed for fertilizers use in agriculture; the limit being in Finland d" 3 mg kg^{-1}. There is currently not enough knowledge available to recommend restrictions on the Cd concentration of wood ash for use as forest fertilizer in Finland. In Sweden, the National Board of Forestry has recommended that it should not be > 30 mg Cd kg^{-1} ash (Arvidsson 2001) and the Danish Environmental Protection Agency recommended that maximum Cd level should be 15 mg Cd kg^{-1} ash and the load should not exceed 0.5 t ha^{-1} per 10 years (Pedersen 2003).

The adsorptive capacity of ash has been used to adsorb Cd from contaminated water (Iyer and Scott 2001, Ricou-Hoeffer et al. 2001). There have also been tests done to remove Cd from wood ash (Pedersen 2003, Pedersen et al.

2003). This electrodialytic process removed about 70% of the Cd (28 mg kg^{-1}) from wood ash but the technology is still at a too early stage to make reasonable cost calculations, but the aim is to make the costs competitive with the costs for land filling. Another way to reduce the amount of Cd in wood ash is to concentrate it in a fine ash fraction (e.g. filter fly ash or condensation sludge), which could be separated from that part of the ash used for fertilization. This could be achieved by new furnace technology (Narodoslawsky and Obernberger 1996, Obernberger 1998).

1.4 Overall conclusion

The use and benefits of wood ash as a forest fertilizer depends on its quality, in particular unburnt carbon, nutrient, heavy metal and organic compound contents. If the wood ash contains high concentrations of harmful substances, it should not be used at all. Because elemental concentrations in wood ash show great variation its quality should always be determined. Where wood ash is used in upland forests it should preferably be supplemented with N in order to maintain a balanced nutrient status. To avoid potential drastic pH effects of wood ash, hardened rather than not loose ash should be used.

2 AIMS OF THE THESIS

My main objective was to find out if the cadmium (Cd) content of wood ash cause harm to boreal, coniferous forest soil humus layer microbes (I-III, VI). In addition, the long-term effects of wood ash on forest soil humus layer microbes (IV) and decomposition rate of needle litter and thereby on tree growth (V) were assessed. Also the potential of wood ash as a remediation agent of heavy metal polluted soil was studied (VII). More precisely, the questions that I have tried to answer were:

1) Is Cd-containing wood ash harmful to forest soil microbes? (I, II, III)

2) Does wood ash Cd transfer to food chains? (III)

3) Does wood ash exposure to simulated acid rain change the bioavailability of Cd and thereby affect forest soil microbes? (VI)

4) Does the form (loose vs. hardened) and the application dose of wood ash affect the magnitude of response of the measured forest soil chemical and microbial variables? (IV)

5) Does wood ash fertilization affect the forest soil microbes and needle litter decomposition rate still after 18-20 years of the treatment? (IV, V)

6) Is wood ash a potential remediation agent for acidified and metal polluted forest soil? (VII)

3 MATERIALS AND METHODS

3.1 Experimental designs

The experimental designs are presented in Table 3 and the details are given in papers I-VII. The wood ash used in studies I-III, IV (experiment 1), VI and VII, was fly ash from burning of softwood (26%) and hardwood bark (66%). Fiber and mineral containing waste sludge (8%) from wastewater cleaning process of paper and pulp mill was also co-burned. A different wood ash was used in the long-term studies IV (experiment 2) and V. The element content of the ashes used in experiments is presented in paper IV.

In papers I and II, I used a laboratory microcosms approach to investigate the combined and separate effects of Cd and wood ash on the humus microflora and the Cd bioavailability. In addition, the effect of different forms (water-soluble $CdCl_2$ and insoluble CdO) and application levels of Cd (0, 400 and 1000 mg kg^{-1} ash or pumice) were studied.

The objectives of paper III were to test in field conditions, if the Cd of wood ash has the potential to affect the coniferous forest humus microflora and if Cd becomes enriched in the food chain 1-4 years after fertilization. These objectives were tested with wood ash and wood ash spiked with extra Cd (400 mg kg^{-1} ash) applied onto the forest floor. Cd concentrations of different compartments (humus, soil percolation water, mushrooms, fruits and leaves of berries, and needles) of the forest ecosystem were determined.

There were two aims for paper IV. The first aim was to compare the effects of loose and hardened ashes on humus layer microbes in the field 1-3 years after fertilization. This experiment was performed using two application levels of the ashes and repeated in two forest stands of different fertility. The second aim was to study the long-term effects of loose ash in four forest stands of different site fertility 18 years after ash application.

Field study V aimed to examine if the wood ash treatment is reflected in the decomposition rate of Scots pine needle litter and Scots pine stem volumes, and if wood ash affects the quality of the Scots pine needle litter, which is then reflected in the decomposition rate. To achieve these objectives the layout of our experiment was as follows: Scots pine (*Pinus sylvestris*) needle litter samples from control plots and plots that had been fertilized with wood ash 19 years earlier were exposed in a reciprocal experimental design to detect the effects of ash fertilization and needle litter origin on the decomposition rate (Fig. 1 in V). The experimental design was repeated in two Scots pine forest stands of different fertility.

In paper VI, I tried to elucidate if simulated acid rain increases the leaching of Cd from wood ash and does it become bioavailable and harmful to co-

Table 3. Experimental designs in the studies.

Study	Symbol	Ash dose t ha^{-1}	Cd addition mg kg^{-1}	Irrigation[d]	Duration of ash treatment	Study type	Replicates	Site type of study/humus sample forest[e]	Location
I, II[a]	P	0	0	H_2O	2 months	laboratory microcosm	10	EVT	64°43'N/26°02'E
	PLO	"	400	"	"	"	5	"	"
	PHO	"	1000	"	"	"	"	"	"
	PLC	"	400	"	"	"	"	"	"
	PHC	"	1000	"	"	"	"	"	"
	A	5	10	"	"	"	10	"	"
	ALO	"	400	"	"	"	5	"	"
	AHO	"	1000	"	"	"	"	"	"
	ALC	"	400	"	"	"	"	"	"
	AHC	"	1000	"	"	"	"	"	"
III	C	0	0	natural conditions	1-4 years	field	3	"	"
	A	3	15	"	"	"	"	"	"
	ACd	"	400	"	"	"	"	"	"
IV[b]	C	0	0	"	1-3 years	"	"	ECT, VMT	64°43'N/26°02'E, 65°01'N/25°07'E
	A3	3	15	"	"	"	"	"	"
	A9	9	"	"	"	"	"	"	"
	HA3	3	13	"	"	"	"	"	"
	HA9	9	"	"	"	"	"	"	"
	C-18	0	0	"	18 years	"	4	CT, VT, MT, OMT	62°03'N/24°51'E,62°16'N/24°20'E
	A3-18	3	ND	"	"	"	"	"	61°00'N/24°45'E, 61°02'N/24°39'E
V	C	0	0	"	19-20 years	"	"	CT, VT	62°03'N/24°51'E,62°16'N/24°20'E
	A	3	ND	"	"	"	"	"	"
VI	C	0	0	H_2O or H_2SO_4 (=SAR)	3 months	field microcosm	"	MT	60° 07'N/23° 18'E
	A	5	10	"	"	"	"	"	"
	ACd	"	1000	"	"	"	"	"	"
VII[c]	Control	0 or 5	0 or 15	H_2O	2 months	field+laboratory microcosm	5	CT-VT	69°45'N/27°01'E
	Acid	"	"	" +H_2SO_4	"	"	"	"	"
	CuNi	"	"	" +$CuSO_4$+$NiSO_4$	"	"	"	"	"
	CuNi+Acid	"	"	" +H_2SO_4+$CuSO_4$+$NiSO_4$	"	"	"	"	"

[a] P = pumice, O = Cd form CdO, C = Cd form $CdCl_2$. [b] A = loose wood ash, HA = hardened wood ash, study IV comprises short- and long-term experiments, experiment I (1-3 years) and 2 (18 years), respectively; ND = not determined; SAR = simulated acid rain; [c] Humus from field was collected after 9 years exposure to acid and/or metal irrigation. Remediation study with wood ash done in laboratory microcosms; [d] In all studies, the irrigation were done at 2- to 3-days intervals; [e]EV=*Empetrum-Vaccinium*, EC=*Empetrum-Calluna*, VM=*Vaccinium-Myrtillus*, C=*Calluna*, V=*Vaccinium*, M=*Myrtillus*, OM=*Oxalis-Myrtillus*.

niferous humus microbes. The effect of Cd spiked into the ash (1000 mg Cd kg^{-1}ash) in response to the treatments was also determined. The simulated acid rain (SAR; pH 3.1) plots received a sulphur load of 3.64 g S m^{-2}, which was 15 times more than the S deposition on the water irrigated control plots.

In paper VII, I have assessed the effects of moderate amounts of continuous acid and Cu-Ni deposition on humus layer microbial community in the field after nine growing seasons. To determine whether these contaminated soils could be remediated, samples from the field were placed in laboratory microcosms and water irrigation combined with wood ash fertilization remediation treatment was evaluated. Microcosms that were only irrigated with water served as a control. The cumulative S, Cu and Ni load over the period 1991 – 2000 were for the CuNi plots 1220, 160 and 100 mg m^{-2}, and for CuNi+Acid plots 18 860, 160 and 100. The cumulative S load for the Control and Acid plots was 1080 and 18730 mg m^{-2}, respectively. The pH values for the irrigation in Control, Acid and CuNi plots were 5.5, 3.1 and 5.7, respectively.

3.2 Samplings

In all the studies, samples were taken from the organic, humus (F/H) layer, hereafter referred to as soil samples. At least 21 cores (usually 40 mm in diameter) were taken; that is, seven cores from three lines, and then combined to form one composite, bulk sample. The samples were sieved (2.8 mm mesh), visible plant material was removed, and then stored at +4°C before analyses were conducted.

In paper III, the wood ash was spread on the study plots in autumn 1997. Soil samples were collected in August 1998, November 1999, September 2000 and 2001. In addition needle (*Pinus sylvestris* L.), berry (*Empetrum nigrum*, *Vaccinium uliginosum*, *Vaccinium vitis-idaea*) and mushroom (*Lactarius rufus*) samples were collected. *P. sylvestris* needles from the previous year were collected each autumn, always from eight trees and bulked to make composite sample for the plot. Unfortunately, it was not possible to take an adequate mushroom and berry sample each year, that is, 6-20 mushroom specimens (upper half of stipe and cap) and 1.5 decilitres of berries per sample plot. Soil water was sampled with suction-cup lysimeters installed at depths of 5 and 20 cm below the soil mineral layer surface six times during the snow free period in 1998 and 2001, and seven times in 1999 and 2000. In 2000 also the leaves of *V. uliginosum* and *V. vitis-idaea* were collected from all over each study plot to form a composite sample of ca. two decilitres (over 2 g dry matter). Litterbags for mass loss determination were sampled in November 1999, 2000 and 2001 after 2, 3 and 4 years exposure in the field, respectively.

In paper IV, the wood ash in the short-term study (experiment 1) was spread in May-July 1997 and soil samples collected in August 1998, June 1999 and

September 2000. In the long-term study (experiment 2), the wood ash was spread in July-August 1982 and soil samples collected in September 2000.

In paper V, the needle litterbags were retrieved in autumn of 2001 and 2002 after 4 and 16 months exposure in the field, respectively. Scots pine stem volumes were measured in autumn 1998 (ash was spread in July-August 1982).

3.3 Methods

The various methods used are listed in Table 4. A brief summary is given here; for details refer to papers I-VII. Soil pH was measured in a water suspension. Soil dry matter weight was determined after drying at 105°C and organic matter content determined after furnacing subsamples at 550°C. Total organic carbon and nitrogen content were determined by dry combustion. Total and extractable contents of elements were determined with ICP-AES. In paper III, dissolved organic carbon (DOC) concentrations in soil water was determined with a TOC analyser and NH_4^+ and NO_3^- concentrations determined with a flow injection analyser. Concentrations of Ca and Cd in soil water were analysed by ICP-AES. After collection of the mushroom, berry, leaf and needle samples (III), they were cleaned, air dried and concentrations of Ca and Cd determined by ICP-AES after wet digestion (HNO_3+H_2O_2).

Bioavailability of Cd (extraction with deionized water) and Cu (extraction with 50 mM $CaCl_2$) to bacteria in the air dried soil were determined with two biosensor bacteria that emit light specifically in the presence of Cd or Cu. The enumeration of culturable bacteria was done on agar nutrient plates. Colony forming units (cfu) were counted after 12 days of incubation in darkness at room temperature. Bacterial Cd tolerance was also estimated on nutrient agar plates to which 5 or 20 mg Cd per liter (as $CdCl_2$ x 2.5 H_2O) was added.

Microbial activities measurements were basal respiration, [^{3}H]-thymidine incorporation and needle mass loss rates. Basal respiration rates were determined as the amount of CO_2-C evolved over 23-26 h. Bacterial growth rate and Cd tolerance were determined using the [^{3}H]-thymidine incorporation technique. For the bacterial growth rate, the bacterial cells were extracted from soil, after sample homogenization and centrifugation. In the Cd tolerance assay, different amounts of $CdCl_2$ were added to the bacterial suspension. The Cd concentration (mM) giving a 50% (or 30%) reduction in [^{3}H]-thymidine incorporation was calculated (IC_{50} or IC_{30}). The higher the IC_{50} value, the greater is the tolerance of the bacterial community to Cd. In the determination of the mass loss rate of needles in the papers III and V, the contents of needles remaining in the litterbags were weighed.

The microbial community profiling analyzes were made using the Biolog®, phospholipid fatty acid (PLFA) method, and 16S or 18S ribosomal DNA (rDNA) targeted single step polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). The Biolog® technique used in paper I deter-

Table 4. Methods used in this thesis.

Analysis	Method	Used in study	Reference
Total Cd in humus [a]	wet digestion, HNO_3+H_2O_2 extraction	I, II, III, VI	Tamminen and Starr 1990
Extractable Cd in humus [b]	1.0 M CH_3COONH_4 extraction (pH 7.0) [c]	I, III, VI	Tamminen and Starr 1990
Bioavailability of Cd to bacteria	Bioluminescence of *Bacillus subtilis* BR 151/pTOO24	II, III, VI	Young et al. 1969, Tauriainen et al. 1998
Bioavailability of Cu to bacteria	Bioluminescence of *Pseudomonas fluorescens* DF57-Cu15	VII	Tom-Petersen et al. 2001
Microbial activity	CO_2 evolution	I, III, IV, VI, VII	Pietikäinen and Fritze 1995
Mass loss of needles	litter bags	III, V	
Bacterial growth rate	[^{3}H]-thymidine incorporation	I, III	Bååth 1992a, Kiikkilä et al. 2000
Cd tolerance of bacteria	[^{3}H]-thymidine incorporation	I, III	Bååth 1992b, Kiikkilä et al. 2000
Cd tolerance of bacteria	cfu	I	
CLPP	Biolog EcoPlates	I	Insam 1997
Microbial community structure	PLFA pattern	I, III, IV, VI, VII	Frostegård et al. 1993b, Pennanen et al. 1999
Bacterial community structure	PCR-DGGE, primer pair F984GC+R1378	VI	Heuer et al. 1997
Fungal community structure	PCR-DGGE, primer pair FF390+FR1GC [d]	II, VI, VII	Vainio and Hantula 2000, Pennanen et al. 2001
Total microbial biomass	PLFA analysis	I, III, IV, VI, VII	Frostegård and Bååth 1996
Bacterial biomass	PLFA analysis	I, III, IV, VI, VII	Frostegård and Bååth 1996
Fungal biomass	PLFA analysis	I, III, IV, VI, VII	Frostegård and Bååth 1996
Culturable bacteria	cfu	I	

For the closer description of materials and methods, see papers I-VII

[a, b)] Other elements (Ca, Mg, K, Al, Cu and Ni) were also analyzed with these methods; [c)] In study VI, 0.1 M $BaCl_2$ extraction was used; [d)] In study II primer pair NS1+FR1GC was also used; cfu = colony forming units; CLPP = community level physiological profiling; PLFA = phospholipid fatty acid; PCR = polymerase chain reaction; DGGE = denaturing gradient gel electrophoresis; F = forward primer, R = reverse primer, GC = G+C rich sequence attached at 5´ end.

mines the community level physiological profile (= CLPP), that is, substrate utilization potential, of the bacterial community. The EcoPlates used contained 31 different carbon sources and they were inoculated with 10^{-3} diluted soil suspension. The EcoPlates were incubated at 20°C and the absorbances read every day until the 120 h was reached. The PLFA method was used to detect changes in microbial community structure. Lipids were extracted from the soil with a one-phase mixture (1:2:0.8 v/v/v) of chloroform, methanol and citrate buffer (0.15 M, pH 4.0), and fractionated into neutral, glyco- and phospholipids on a silicic acid column. By using nonadecanoate (19:0) as an internal standard the total amount of PLFAs in the soil sample were calculated and this value used as an indicator of soil total microbial biomass ($PLFA_{tot}$). The sum of PLFAs 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 used as an index of bacterial biomass ($PLFA_{bact}$) and the quantity of 18:2ω6 was used as an indicator of fungal biomass ($PLFA_{fung}$). The Biolog® and PLFA methods target mainly bacteria. Treatment-induced changes in fungal community were detected using fungal 390 (II, VI, VII) or 1650 (II) basepair (bp) rDNA fragment (18S) DGGE patterns. Bacterial 434 bp rDNA fragment (16S) DGGE patterns were also used to detect changes in bacterial community in paper VI. The advantage of both the PCR-DGGE and PLFA methods is that they do not rely on the culturability of the microbes.

3.4 Data analyses

Analysis of variance (ANOVA) was used to compare the effects of different treatments. In ANOVA the differences between variable means were tested using Tukey's (II, V) or LSD (III, IV, VI) test. Differences between means were considered statistically significant when $p < 0.05$ (I, II, V, VI) or $p < 0.10$ (III, VII). The mol % and area % values from the PLFA (I, III, VI, VII) and Biolog® data (I), respectively, were standardised by dividing by the standard deviation (correlation matrix) before being subjected to principal component analysis (PCA). In paper IV, the PLFA pattern was explored with global non-metric multidimensional scaling (MDS) (Clarke 1999). In paper VI MDS was used to analyse the data matrix of DGGE gel bands. In papers II and VI the Kruskall-Wallis non-parametric ANOVA followed by a mean ranks test was performed for the biosensor data because the assumptions of normal distribution and equality of variances were not met. Canonical correlation analysis (CCA) (Gittins 1985) was used to investigate the relationships between chemical and biological variables (III, IV). The scores from PCA and CCA were also tested with ANOVA. The data were log-transformed where necessary. Pearson correlation tests were used to evaluate the relationships between some individual variables. The software used for ANOVA, PCA and CCA were Statistix 7 (Analytical Software 2000), PC-ORD version 4 (MjM Software Design 1999) and SAS (Institute Inc. 1996), respectively.

4 RESULTS AND DISCUSSION

4.1 Cd effects on forest soil microbes

The addition of Cd to the soil can disturb the nutrient cycling of forest ecosystems because of its potential toxic effects on microbes. Cadmium amendments have been shown to inhibit phosphatase, sulphatase and respiration activities (Speir et al. 1999), change microbial community structure (Frostegård et al. 1993b) and reduce microbial biomass (Frostegård et al. 1993b) and dehydrogenase activity (Welp 1999). Cadmium concentrations as low as 1-5 mg Cd kg^{-1} humus have been found to inhibit the activity and change the community structure of forest soil microbes (see review by Bååth 1989). Microbes have been shown to have a number of mechanisms to prevent Cd toxicity, including the exclusion of Cd by an energy-dependent pump situated in the cell membranes of resistant stains and the production of Cd-binding proteins in microbes that accumulate Cd (Trevors et al. 1986).

In order to estimate the possible effect of Cd not associated with ash, Cd with pumice was spread onto the soil (I, II). Pumice proved to be an ideal agent to distribute the small amounts of Cd evenly over the soil surface, because it has no effects on the microbes or pH of the soil compared to untreated soil (I). The different forms of Cd (CdO vs. $CdCl_2$) had no effect on the soil respiration rate, but the level of Cd applied (0, 400 or 1000 mg kg^{-1} pumice) influenced the respiration rate. The highest application of Cd (5 t pumice ha^{-1} of 1000 mg Cd kg^{-1} pumice) decreased respiration rates compared to lower Cd levels (I; Fig. 1a, page 34). The form and level of Cd had no effect on the bacterial growth rates, bacterial and fungal biomasses, culturable bacteria, tolerance of the bacterial community to Cd measured with thymidine incorporation or with a more conventional cfu method (I). Neither did the substrate utilisation pattern of bacterial community (I; Biolog®) nor fungal community structure (II; PCR-DGGE) change.

When Yrjälä et al. (2004) analyzed the *Archaea* community from paper I microcosm soil with the PCR-DGGE method, they detected no Cd effect. Neither did Cd have an effect on community structure of *Actinomycetes* (unpublished result). Díaz-Raviña et al. (1994) tested the development of community tolerance to Cd (added as $CdSO_4$) in agricultural soil over a 5- to 8-month incubation period. The addition of 896 mg Cd kg^{-1} dry soil resulted in the development of a Cd-tolerant community, whereas the addition of 448 mg Cd kg^{-1} dry soil gave minor differences compared to the unpolluted control. In paper I the addition of Cd with pumice to the soil surface at the highest doses, resulted in the amount of Cd to 171 mg kg^{-1} and 182 mg kg^{-1} soil for CdO and $CdCl_2$, respectively. These levels did not exceed the threshold level needed to induce tolerance of the bacterial community to Cd.

The addition of Cd with pumice altered the microbial community structure as detected by the changed PLFA pattern (I; Fig. 1a, page 34). The ANOVA performed on the PC1 scores of principal component analysis (PCA), using the PLFA data, revealed that the high Cd (added as $CdCl_2$) treatment differed significantly from the other treatments, including the control, and the scores correlated significantly ($r = 0.58$) with the total amount of Cd in the soil. The relative mol % of the methylated PLFAs increased with increasing Cd concentration. Fatty acids with a methyl group in the tenth carbon atom from carboxyl end of the chain (10Me16:0, 10Me17:0, 10Me18:0) are found exclusively in actinomycetes (Kroppenstedt 1985). Thus, the result of paper I suggests that Cd application caused a relative increase in the number of actinomycetes. In contrast, the relative mol % of PLFAs 18:2w6 and 20:4, which are found in fungi (Frostegård and Bååth 1996, Stahl and Klug 1996), decreased as a result of Cd addition. The changes observed in the PLFA pattern of the pumiced samples due to Cd addition are consistent with the results of Frostegård et al. (1993b). At the beginning of a 6-month incubation they added solutions of $CdSO_4$ to humus layer samples to give comparable Cd concentrations (56, 112 and 224 mg kg^{-1} dry humus) to those used in study I (≤ 182 mg kg^{-1} dry humus).

In all soil samples treated with Cd spiked pumice, the *Bacillus subtilis* biosensor detected an increased amount of bioavailable Cd when compared to the unspiked pumice controls (II; Fig 1a, page 34). The biosensor detected significantly higher Cd amounts in the soil of the PHC and PHO treatments than in the PLC and PLO treatments. The highest amount of bioavailable Cd (20.2 ± 1.0 (SE) mg Cd kg^{-1} soil) was detected in the PHC treatment. The percentage of bioavailable Cd in soil was only 4.7–11% of the total Cd content. In general, a higher availability of Cd was detected in the soil samples that received the water soluble form of Cd, but the difference between PHC and PHO, and between PLC and PLO, were statistically insignificant.

In conclusion, both Cd application levels (400 and 1000 mg Cd kg^{-1} pumice) increased the amount of bioavailable Cd to bacteria, and highest Cd application level (both CdO and $CdCl_2$) reduced the microbial mineralization activity measured as respiration rate. In addition, the highest application of water soluble Cd changed the microbial community structure measured with PLFA method.

4.2 Wood ash effects on forest soil microbes

The application of ash increased soil pH (I-IV, VI-VII). The pH effect of the ash increased microbial activities as measured by soil respiration (I; Fig. 1a, III; Fig. 1b, IV, VI, VII) and thymidine incorporation rates (I, III). These results are in accordance with investigations where higher soil respiration (Bååth and Arnebrant 1994, Fritze et al. 1994b, Khanna et al. 1994, Fritze et al. 1995) and

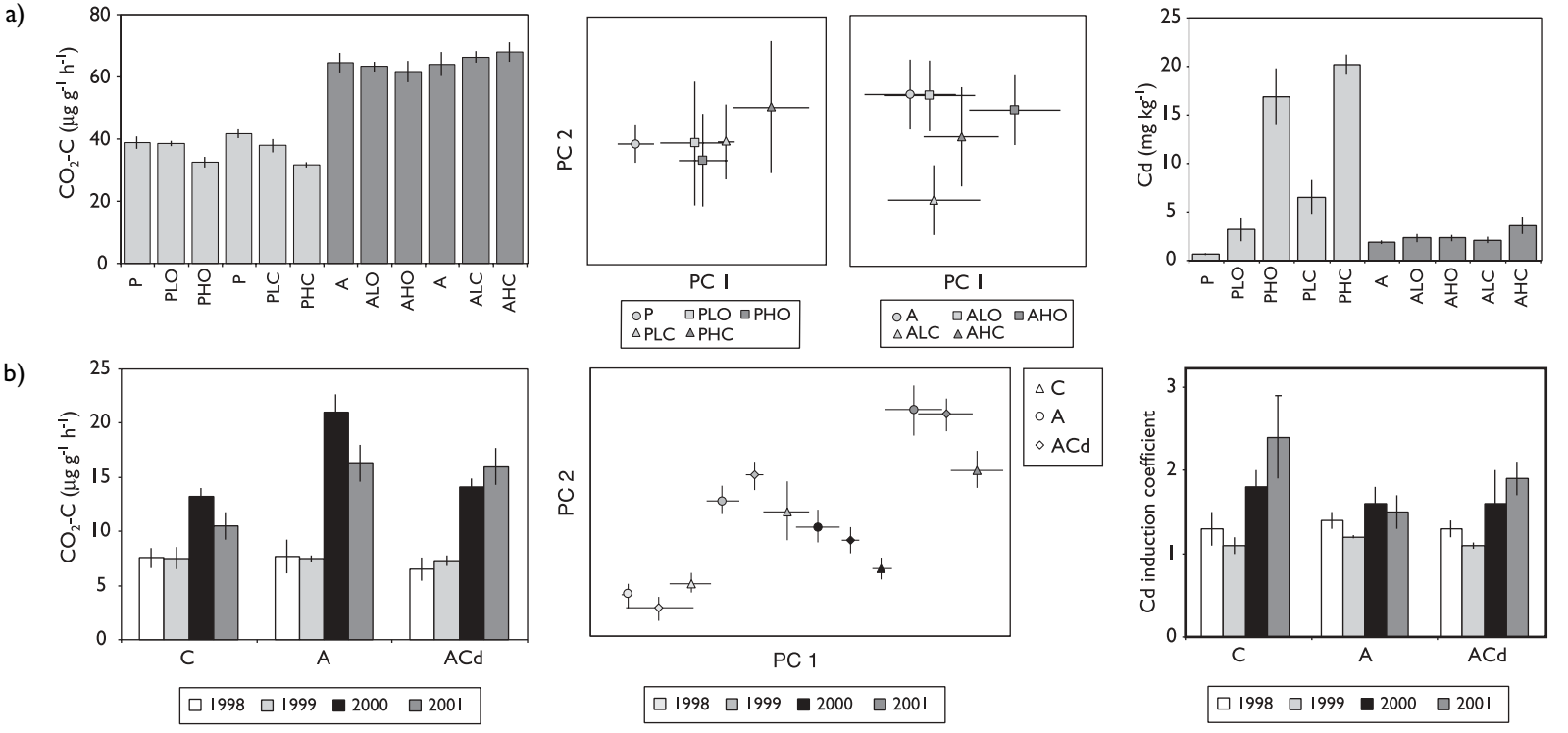

Fig. 1. Respiration rates, PLFA patterns and reactions of Cd-biosensors in the studies I-II (a) and III (b). In study III, the majority of samples exhibited bioavailable Cd-levels below the reliable quantification level and the induction coefficients were not transformed to Cd concentrations. Bars indicate standard error. See Table 3 for treatment symbols.

thymidine incorporation rate (Bååth and Arnebrant 1994, Bååth et al. 1995, Hagerberg and Wallander 2002) were detected after ash fertilization. According to Khanna et al. (1994), much of the respired C is derived from soil sources rather than the C added in the ash. In many studies, wood ash fertilization resulted in increased soil water dissolved organic carbon (DOC) concentrations (see chapter 1.2.1), which could act as a source of carbon for microbes and explain the rise in respiration and thymidine incorporation.

In accordance with the activity measurements, the number of culturable bacteria also increased (I). Similar increase in cfu values due to wood ash fertilization at levels of 5 t ha^{-1} have been reported for coniferous forest humus by Bååth and Arnebrant (1994). The increased microbial activity of the ash treated samples was also accompanied by a change in the bacterial substrate utilisation pattern (I; Biolog®), and in the microbial (I; Fig. 1a, III; Fig. 1b, IV, VI, VII; PLFA), bacterial (VI; PCR-DGGE), fungal (II, VI; PCR-DGGE), archaeal (Yrjälä et al. 2004) and actinomycetal (unpublished result) community structure.

The Biolog® results revealed that the bacterial community in the ash treated soil was able to use other C sources compared to the bacteria community in the pumice treated samples (I). The ash application was therefore great enough to change the microbial community structure (PLFA) to such an extent that different subsets of the bacterial community became enriched in the Biolog® plate, being capable of utilizing different C-sources than the subsets of the untreated or pumiced soil samples (I).

A change in the PLFA pattern due to increased humus pH has been demonstrated earlier in an area subjected to alkaline pollution (Bååth et al. 1992), forest liming (Frostegård et al. 1993a) and wood ash fertilization (Frostegård et al. 1993a, Bååth et al. 1995). In a 3-year field mesocosm study by Liiri et al. (2002b), wood ash treatment changed the microbial PLFA and bacterial substrate utilization pattern. Changes in humus quality with increasing ash fertilization levels have been measured using infrared spectroscopy (Bååth et al. 1995). These authors showed that the humus quality changed but were only partly successful in their attempts to correlate the changes in the PLFA pattern with soil pH or substrate quality. Consequently, they concluded that changes in the PLFA pattern of the soil organisms were related to an altered substrate quantity (availability of substrates), quality and pH.

The results from paper VII showed a slight increase in the abundance of actinomycetes (indicator PLFAs 10Me16:0, 10Me17:0 and 10Me18:0 see chapter 4.1) and decrease that of fungi (indicator PLFA 18:2ω6) following ash fertilization. Similar changes in fungal and actinomycetal PLFAs after wood ash treatment in the microcosm experiment were found in paper I. In the field studies by Frostegård et al. (1993a), Bååth et al. (1995), III and IV, the amount of indicator PLFA (16:1ω5) for arbuscular mycorrhiza (AM) fungi increased after ash application. In these field studies the amount of PLFAs 10Me16:0 and 18:2ω6 either decreased or did not change, and that of PLFAs 10Me17:0 and 10Me18:0 either increased or did not change. However, in the long-term study

IV (experiment 2) the abundance of fungal PLFA increased, and that of PLFAs 10Me16:0 and 10Me17:0 decreased in the wood ash treated plots. The most striking difference between the results from these field studies and microcosm studies I and VII was that the amount of the fatty acid 16:1ω5 decreased after ash amendment in microcosm studies. One explanation for this difference may be related to the absence of plants in microcosms. Herbs and grasses thrive after ash fertilization (see chapter 1.2.2) and the amount of fatty acid 16:1ω5 increases when the amount of herbs and grasses increase (Pennanen et al. 1999, Saetre and Bååth 2000).

The results of bacterial and fungal biomass measurements in this thesis, were not consistent with each other. Mean bacterial (I, IV (experiment 1); $PLFA_{bact}$) and fungal (I, IV (experiment 1), VI; $PLFA_{fung}$) biomasses either decreased or did not react to wood ash treatment (VI; $PLFA_{bact}$, III, IV (experiment 2),VII). These kinds of contradictory results have also been observed by others. For example, Bååth et al. (1995) observed a decrease in bacterial and fungal biomasses in a field study with ash treatment, but Frostegård et al. (1993a) did not find a significant ash effect on bacterial and fungal biomasses.

In papers II and VI, fungal 18 S rDNA primer pair detected an ash effect but in paper VII no ash effect on fungal community was detected. It can be argued that at the time of sampling these fungal species were the most dominant growing. The fungal species diversity as indicated by spores is probably not detected by the PCR-DGGE method. In a soil microfungal study (Fritze and Bååth 1993), the fungal species composition growing on agar plates was not different if the isolation was performed from vegetative hyphaea or from the spores. Microcosms in papers II, VI and VII did not include plant seedlings and, thus mycorrhizal fungi were probably excluded, leaving only saprophytic fungi that could be detected. Using isolation techniques it has been shown that ash fertilization (Bååth and Arnebrant 1993) and fly ash deposition (Fritze and Bååth 1993) changes the composition of the microfungal flora inhabiting the humus layer. The same conclusion was obtained by using the PCR-DGGE method (II, VI).

Experiment 1 of paper IV compared two forms, loose and hardened wood ash (abbreviations A and HA, respectively) at two fertilization levels, 3 and 9 tons per hectare (these numbers follow the abbreviations). The results showed that ash of both types decreased soil extractable Al, increased soil pH and exchangeable base cation concentrations; reactions to ash amendment that have been widely reported in the literature (see chapter 1.2.1). Hardened ash did not have as strong effects on above-mentioned soil properties as loose ash did. Increase in soil extractable Ca and Mg concentrations was dependent on the dose and form of the ash applied, with A9 treatment having the highest values, followed by HA9 and A3, and then the HA3 treatment (IV; experiment 1).The lowered leaching rate of base cations due to hardening has also been confirmed in laboratory studies by Eriksson (1998b) and Ring et al. (1999). The hardening of ash by granulation helps avoid the rapid dissolution of the ash and thereby avoiding drastic increases in humus pH (Eriksson 1998b), burning

damage to mosses (Kellner and Weibull 1998) and changes in the coverage of fungi and vascular plants (Rühling 1996). However, the nutritional response with hardened, granulated and pelletized wood ash on the forest vegetation was found to be similar to that with loose wood ash (Moilanen and Issakainen 2000, Hytönen 2003).

Due to its slower dissolution rate the effects of hardened ash on soil microbes may be expected to be less than loose ash. Both multivariate statistical approaches supported the above hypothesis. The canonical correlation analysis (CCA) separated the ash treatments according to the form and level of ash used. The vectors that best explained the separation of the treatments were increases in bacterial growth rate, microbial respiration activity, and soil extractable Ca concentration (Fig. 2 in IV). The A9 treatment that has highest values for these variables separated from other treatments. Treatments A3 and HA9, and HA3 and C clustered into same group. The multidimensional scaling (MDS) procedure, using the eluted phospholipid fatty acids (PLFAs) data, separated the A9 treatment from the other treatments from the first year onwards. In the third year, the HA9 and the A3 treatments started to separate from the control and remained separated from the A9 treatment (Fig. 3 in IV).

The aim of experiment 2 in paper IV was to estimate the duration of the ash effect on upland soil microbes. The fact that the microbial community structure (PLFA pattern) and activity (respiration and thymidine incorporation rates) were still different from the control after 18 years of ash fertilization points toward a very long-term effect of wood ash on soil microbes. However, there were no clear long-term ash effect on the biomass indicators except that the $PLFA_{fung}/PLFA_{bact}$ ratio was slightly higher in the ash treated (A3-18) than in the control plots (C-18). The CCA separated the old ash plots according to microbial activity (respiration and thymidine incorporation rates) and soil extractable Ca concentration that are the same variables as in the short-term field experiments in papers III (see chapter 4.3 below) and IV. These findings provide new information about the longer-term effects of wood ash on upland forest soil microbes. Previous studies have only studied these effects for short time periods of 1-6 years after application.

One of the oldest documented ash fertilization trial in Finland was performed in a forest growing on peat and therefore not directly comparable to study IV. The trial on peat (no treatment replications) showed elevated peat pH (Silfverberg and Huikari 1985), Scots pine stem volume growth and decomposition rate of cellulose strips (Moilanen et al. 2002) after 30, 47 and 50 years of fertilization, respectively, with rather high amounts of loose ash (8 and 16 t ha^{-1}). In study IV humus pH (highest difference between pH of C-18 and A3-18 plots was 1.2 units) was also an important discriminating variable in the CCA. From these results one can speculate that the microbiological effects of loose ash on upland soil can persist for 50 years, and the effects of hardened ash can persist for longer.

The needle decomposition experiment in paper V was performed on the same sites as in paper IV that had been treated with wood ash 19-20 years ear-

lier. The litterbags were retrieved after 4 and 16 months. The loss of needles mass was significantly larger on the ash treated plots than on control plots. The increased microbial activity and the changed microbial community structure shown in paper IV were thus reflected in enhanced needle decomposition. On drained peatland sites, Silfverberg and Hotanen (1989) have observed a similar increase in needle decomposition rate as a result of long-term ash effects. However, in a 4-year long field study III, no significant increase in the mass loss of needles was detected although rise in respiration and thymidine incorporation rates were detected. It is difficult to explain the lack of a wood ash effect on the mass loss of needles. Most likely, the time was not too short for a fertilization effect to occur, because Smolander et al. (1996) found increased mass loss of Scots pine needle litter on study plots fertilized with wood ash (2.5 t ha^{-1}) only 8 months prior to the start of the decomposition experiment. One possible explanation could be the unfavourable quality of needles in study III.

The ash fertilization induced changes in the ecosystem may be reflected in the quality of the coniferous trees needles. In both sites in paper V, needles originating from the ash fertilized plots had lower C-to-N ratios. The origin, and thus the quality of needles, did not significantly influence the decomposition rate although ash needles exposed on ash plots (AA) had a slightly higher decomposition rate than control needles exposed on ash plots (CA) during the first 4 months (Fig. 2 in V). Further details about the quality of needles on the decomposition rate are discussed in paper V.

Scots pine stem volumes increased due to ash fertilization (V). The enhancing effect of ash fertilization on both needle mass loss and tree growth was more pronounced in the less fertile CT than the VT site. This difference was accompanied by a significant increase in concentrations of P in the humus layer of CT site. Phosphorus and especially N deficiency is quite common in Finnish tree stands growing on mineral soils, and B deficiency also sometimes occurs (Raitio et al. 2000). Humus layer N concentrations did not respond to wood ash fertilization and the amount of B was not analyzed. Usually, in contradiction to the findings in study V, the effect of wood ash fertilization on the growth of coniferous trees on mineral soils has been insignificant (see chapter 1.2.3.1). However, Tamminen (1998) did observe an increase in the height growth of 8-15-years old Scot pines 2-5 years after wood ash fertilization (3 t ha^{-1}).

One negative effect following wood ash application was the increase in NO_3^- concentrations in the soil water sampled from the ash treated plots (A) at 0.2 m depth (III). This increase in NO_3^- concentrations could be due to an increase in the nitrification rate in response to the increased soil pH (Martikainen 1984, Khanna et al. 1994, Paavolainen and Smolander 1998, Priha and Smolander 1999). Kahl et al. (1996) and Ludwig et al. (2002) also observed increased NO_3^- leaching after wood ash application on sandy soil. Increased NO_3^- leaching after ash application, however, is not a rule since many other wood ash studies have not detected it (see chapter 1.2.1).

In conclusion, the microbiological variables that responded to wood ash fertilization were: respiration and thymidine incorporation rates, cfu values, substrate utilisation pattern of the bacterial community, and the structure of microbial, bacterial and fungal communities. In some studies, fungal and bacterial biomasses have also decreased. In the field studies, the activity measurements reacted earlier (1-3 years after ash treatment) than community structure changes (3-4 years after ash treatment) and the response did not differ between forest site types. The degree of change on the measured soil chemical and microbial properties depended on the time since application and the level and form of ash applied. Increased soil microbial activity and changed community structure were still detected 18 years after wood ash treatment and Scots pine needles on treated sites decomposed faster 20 years after treatment.

4.3 Wood ash Cd effects on forest soil microbes

Cadmium in unspiked or Cd spiked wood ash had no significant effect on respiration (I; Fig. 1a, III; Fig. 1b) and thymidine incorporation rates (I, III), needle litter weight loss (III), cfu counts (I), total ($PLFA_{tot}$), bacterial ($PLFA_{bact}$) and fungal ($PLFA_{fung}$) biomasses (I, III) or on the substrate utilisation pattern of the bacterial community (I), or microbial (I; Fig. 1a, III; Fig. 1b), bacterial (VI), fungal (II, VI), archaeal (Yrjälä et al. 2004) and actinomycetal (unpublished result) community structure in the ash treated soil samples. Neither did the amount of bioavailable Cd to bacteria in the soil increase (II; Fig. 1a, III; Fig. 1b). The results of microcosm experiments reflected the same trends than the field experiment.

In study II, *Bacillus subtilis* biosensor values of Cd spiked ash treatments did not differ from their unspiked ash controls and the mean level of bioavailable Cd was 2.4 mg kg^{-1} soil in all the ash treatments. Thus, the soil samples receiving pumice spiked with Cd (see chapter 4.1 above) had significantly higher amounts of bioavailable Cd than the ash treated samples. In study III, the total Cd concentrations in wood ash treated soil were about ten times lower than in the microcosms study II and the amount of bioavailable Cd to bacteria were in many samples below a reliable quantification level.

Fritze et al. (1995) have shown earlier that the soil respiration rate decrease when Cd is added to wood ash treated humus. Humus layer samples from a field experiment given 5 t ha^{-1} of wood ash required more than 4000 mg Cd kg^{-1} humus to decrease the respiration rate by 50 %, whereas the unfertilized control humus required 1570 mg Cd kg^{-1} humus to reach the same degree of inhibition (Fritze et al. 1995). Their study indicated that wood ash can to some extent reduce the toxicity of Cd, but levels of Cd given were unnaturally high. The experiment performed in paper I, is in this respect more realistic because the Cd was first mixed with the wood ash to give a theoretical maximum level

of 1000 mg Cd kg^{-1} ash before spreading it onto the surface of the soil to imitate a fertilization load of 5 t ha^{-1}. Converting this to the amount of Cd per soil dry weight used in this experiment, 196 mg Cd kg^{-1} humus entered the soil system. This was enough to reduce the soil respiration rate by 20 % in the absence of ash (see chapter 4.1 above). In the presence of ash, even the highest addition of Cd had no effect on soil respiration (I; Fig. 1a).

Principal component analysis (PCA) of the PLFAs separated, but not significantly, the Cd-treated ash samples from the untreated ash samples along the first PC (I; Fig. 1a). Actinomycetes (representative PLFAs 10Me16:0 and 10Me17:0), which benefited from the addition of ash or Cd alone, suffered from the combined effect of ash and Cd. The third methylated PLFA 10Me18:0 did not react markedly to the Cd treatments in the ash treated soil. Since the differences among the treatments were not statistically significant and the PCA axis 1 scores did not significantly correlate with the measured Cd concentration in the soil, it can be concluded that the presence of ash countered the toxic action of Cd on the microbial community. This was probably due to the capacity of the ash to adsorb the Cd and to increase soil pH. The hypothesis that wood ash can protect bacteria from Cd toxicity was supported by the finding that the treatment with just wood ash did not show the effects on Cd bioavailability, microbial respiration rate and community structure that the Cd-alone treatment had.

Bacterial growth rate measured by the thymidine incorporation method in all ash treated samples was more sensitive to Cd additions than the pumice treated samples (I). On average, only 0.0023 ($\pm$ 9.1 x 10^{-5}) mM Cd (= 0.26 mg Cd l^{-1}) was needed to decrease the thymidine incorporation rate by 30%, whereas for the pumice treated samples 0.21 ($\pm$ 2.6 x 10^{-2}) mM Cd (= 24 mg Cd l^{-1}) was required. This result indicated that the bacterial community of the ash treated soil, which was isolated into the water solution before the addition of Cd in the tolerance test, was more sensitive to external Cd than the respective bacterial community of the pumice treated controls. In the field study III, cadmium tolerance IC_{50} values indicated an ash but not a Cd effect two and three years after wood ash addition. Indeed the bacteria in control soil were more tolerant to Cd than bacteria in the ash treated (A and ACd) soils. Thus, according to thymidine incorporation method, ash treatments did not induce bacterial tolerance to Cd. The bacteria that could be cultivated on nutrient media, however, reacted in the opposite (I). The addition of 5 mg Cd to 1 liter of nutrient agar decreased the cfu of the pumice treated samples by nearly 100%, whereas the cfu of the ash treated samples decreased by between 20 to 70%. The traditional plate count method supports the hypothesis that ash can protect bacteria from Cd toxicity. There are many possible reasons for the discrepancy between the results given by these two methods. One of them could be the different incubation times with external Cd that were used, 12 days in the plate count method and 2 hours in the thyminidine incorporation method. Thus, the thymidine incorporation method detects Cd-tolerance of fast growing bacteria and plate count method of slower growing bacteria. One can hy-

pothesise that the decreased IC_{50} value may have been due to increases in bacterial growth rather than a decreased tolerance, since rapid growing bacteria are, in general, more sensitive to external disturbances.

In paper III, the chemical and microbiological data were summarised using a multivariate statistical approach (Canonical Correlation Analysis, CCA; Fig. 1 in III). Accordingly the A and ACd treatments started to separate from controls, but not from each other from the third year onwards. The higher Cd additions in the ACd treatment did not produce any effect on the microbiological and chemical variables differing from that produced by the ash only (A). This is verified by the fact that neither total nor extractable Cd levels were selected as key variables in the formation of the CCA. In contrast, the increase in soil pH, respiration and thymidine incorporation rates and concentrations of total and extractable Ca were selected as key variables that best explained the separation of treatments as they all increased due to wood ash treatment. In addition, the changes in PLFA pattern caused by wood ash affected the formation of the CCA.

In general, the liberation of Cd from different kind of ashes is low (Eriksson 1998b, Ring et al. 1999, Steenari et al. 1999, Richards et al. 2000, Ramesh and Koziñski 2001, Praharaj et al. 2002, Nieminen 2003). A laboratory study showed that solution pH has to be lower than 4.0 before Cd is released from wood ash (Hansen et al. 2001). Zhan et al. (1996) showed that, at solution pH values lower than 6.0, which are normal for rain water, there is a sharp increase in the amount of dissolved Cd from wood ash. Holmberg et al. (2000) showed that almost 15% of the Cd in wood ash granules leached during a 7 months exposure in the field. In paper III, the ACd and A treatments respectively added 128 and 4.5 mg Cd m^{-2} to the forest floor, which theoretically could leach when the ash-induced increase in pH effect stops and pH declines. In the last sampling, that is four years after wood ash treatment, soil pH was 5.6 in A and 5.5 in ACd plots (III).

Little is known about the effect of acid rain on microbial function and community structure of the humus layer fertilized with wood ash. As acidic deposition is still a threat to the terrestrial environment (Alewell et al. 2000) and this could affect the liberation of Cd from the ash when applied to the forest. In paper VI, watering the microcosms in the field with simulated acid rain (SAR pH 3, H_2SO_4 acid, total 3.64 g S m^{-2}) over the whole growing season decreased the soil pH but did not change the results achieved with water irrigation, that is, change in microbial, bacterial and fungal community structures due to wood ash application. Although there was probably a higher dissolution rate of the ash due to the SAR treatment, Cd in the wood ash was not liberated into bioavailable forms as measured with the biosensor *B. subtilis*. The higher dissolution of the ash induced a higher basal respiration rate.

Neither the Cd occurring naturally in wood ash nor that added to wood ash, had any effect on the measured microbial variables. In addition, irrigation with SAR and the level and form of added Cd had no effects. In conclusion, wood ash protected the microbes from the harmful effects of Cd.

4.4 Wood ash Cd transference into food chains

Cadmium may affect human health through carcinogenity, renal and bone effects, and diet is the main source of human exposure to Cd. There is a potential risk that Cd from wood ash enters into the human food chain directly through the eating of mushrooms and berries or indirectly through eating game animals. Ground water contaminated with Cd may also be a source.

The soil total and extractable Cd concentrations in the ACd plots increased, but the increases were not reflected in Cd concentrations in the soil water collected at 5 and 20 cm depth below the soil mineral layer surface or in the Cd concentration of berries and leaves of *Vaccinium uliginosum* and *Vaccinium vitis-idaea* or *Pinus sylvestris* needles (III). In the study by Bramryd and Fransman (1995), wood ash fertilization did not increase EDTA-extractable Cd in the humus or mineral soil layers 10 years after application. A similar result was obtained by Arvidsson and Ludkvist (2003) 6 years after ash application. Wood ash treatment was also shown to not increase Cd concentrations in soil water (Ring et al. 1999), runoff water (brook) (Tulonen et al. 2002), ground water (Piirainen 2001) nor in Norway spruce needles (Arvidsson and Lundkvist 2002).

Higher Cd concentrations in the mushroom *Lactarius rufus* and in the berries of *Empetrum nigrum* growing on ash spiked with Cd treated plots was found in paper III. The Cd concentrations in *L. rufus* correlated with soil total ($r = 0.90$) and extractable ($r = 0.84$) Cd concentrations. This indicated that Cd could enter the human food chain. Even though the ACd treatment added 28 times more Cd to the soil than the A treatment, the Cd concentration in *L. rufus* was roughly only doubled.

There are few studies concerning Cd concentrations in edible mushrooms and berries after wood ash fertilization. The results from paper III described above are in accordance with the few such studies where the Cd concentrations in mushroom *L. rufus* (Rühling 1996, Moilanen and Issakainen 2000, Lodenius et al. 2002) and in berries (Silfverberg and Issakainen 1991, Levula et al. 2000, Rühling 1996, Moilanen and Issakainen 2000, Nilsson 2001) did not increase after wood ash treatment. Moilanen and Issakainen (2000) found, however, that Cd concentrations in *L. rufus* growing on one upland site 19 years after wood ash fertilization increased and on one peatland site it decreased significantly. Lodenius et al. (2002) reported an increase in Cd concentrations in mushroom *Russula emetica*, which is mordant but edible like *L. rufus*, 2 years after the wood ash treatment. Moilanen and Issakainen (2000) reported no change in Cd concentrations in the popular edible mushroom *Russula paludosa* (14 months after ash treatment) and *Lactarius trivialis* (13-52 years after ash treatment) on peatland.

In conclusion, wood ash fertilization did not increase the Cd concentrations in mushroom, berries or in soil water.

4.5 Wood ash as a potential remediation agent for acidified and metal polluted forest soil

Remediation of polluted soils aims to remove or immobilize the pollutants, making them less mobile and available for plants and microbiota. Because wood ash seemed to render the Cd added in wood ash unavailable (II, III, VI), the study VII aimed to test if ash has the same effect on other heavy metals (Cu and Ni). To determine whether samples of humus layer that had been exposed to continuous acid and Cu-Ni deposition for nine growing seasons could be remediated by treatment with wood ash, a microcosm laboratory experiment was carried out. Microcosms that were only irrigated with water served as a control. The wood ash treatment removed all the acid (decrease in pH and base saturation and increase in extractable and bioavailable copper concentrations (Fig. 2), and change in microbial PLFA pattern; (Fig. 1 in VII)) and metal (increase in extractable nickel concentrations, and change in microbial PLFA and fungal DGGE patterns) treatment effects observed in the field. The detoxifying effect of ash on Cu may be due to several mechanisms and they are speculated about in article VII.

In conclusion, it may be possible to use wood ash to remediate acid and metal polluted upland forest sites. Its use should be tested on the polluted soils in the surroundings of industrial smelting plants.

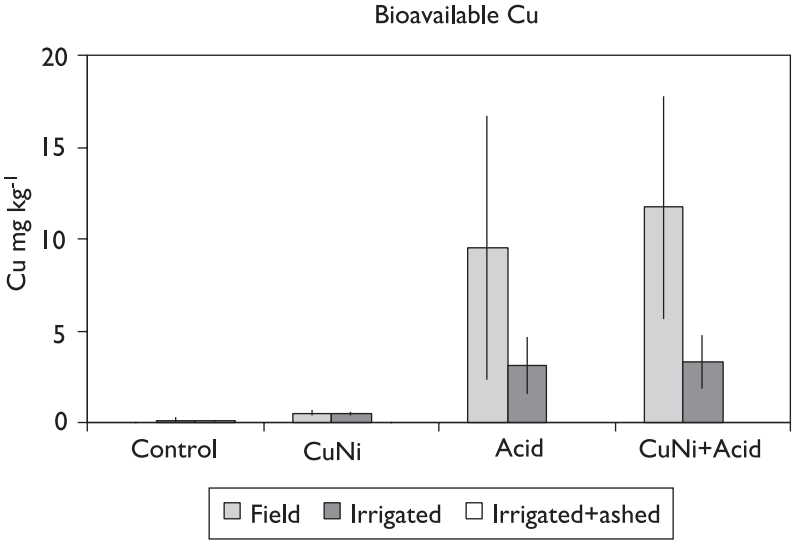

Fig. 2. The amount of bioavailable copper measured with the Cu-sensor *Pseudomonas fluorencens* of the study plots. Bars indicate standard error. See Table 3 for treatment symbols.

5 CONCLUSIONS

Spiking wood ash with Cd at levels that exceed the natural concentrations did not cause harm to forest soil microbes, although Cd addition without ash did. In addition, Cd added with wood ash was not in the bioavailable form, but part of the Cd added without ash was bioavailable to bacteria. Ash thus counteracted the toxic effect of Cd. It can therefore be concluded that the much lower Cd content of the wood ash itself would have no harmful effect on humus layer microflora. A potential risk for Cd to enter the human food chain is through uptake and concentration in mushrooms and berries which was only observed when Cd was added to the ash at unnaturally high levels. The effects of wood ash on soil microbes appeared to be long lasting, with increased needle litter decomposition still detectable 20 years after ash fertilization. The hardening of ash decreased its dissolution rate and therefore hardened ash effects on soil chemical and microbial variables were not as strong as loose ash. Increasing the rate of ash dissolution with simulated acid rain did not increase the bioavailability of Cd to bacteria in the humus layer. The benefits of wood ash fertilization (increase in soil pH and mineral nutrient contents) to microbes, plants and trees far outweigh any risk of the Cd in the ash having harmful effects on the humus microflora or entering the human food chain. However, since wood ash contains between 1 – 30 mg Cd kg^{-1} ash, it would be prudent not to apply ash more than once during a tree generation in the same site, as there remains the possibility that the Cd could be released after the effect of ash on pH levels off. In addition, wood ash can be used for remediation of acid and metal polluted humus due to its capacity to adsorb metals and increase soil pH.

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