Stockholm University

Master Thesis

Degree Project in Geochemistry 60 hp

The influence of microbial communities on the chemical properties of podzolic soil

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Stockholm 2013

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Sammanfattning

Mikroorganismer är allestädes närvarande i jorden och genom sin påverkan på omgivningen så spelar de en viktig roll i kretsloppet av näringsämnen, varigenom de indirekt påverkar näringstillgängligheten för växter. För att utveckla kunskaperna kring hur mikroorganismers inverkan på jordmiljön ser ut så genomfördes ett tre månader långt inkubationsexperiment där de mikrobiella samhällena tillhörande O-, E-, B- och C-horisonten i en podsol extraherades och återinokulerades i steriliserad jord från varje horisont. Detta gjordes för att testa hur de mikrobiella samhällena skulle påverka de olika horisonterna samt för att undersöka om de olika extrakten skulle ha olika påverkan på jordens kemiska egenskaper. Experimentet var även designat för att studera det eventuella förhållandet mellan den mikrobiella aktiviteten och jordens kemiska egenskaper. För att testa vilken effekt de fyra extrakten kunde ha på jord så analyserades följande parametrar innan och efter experimentet: pH; totalt löst organiskt kol, totalt löst kväve och fosfor; lösta organiska syror med låg molekylvikt och sideroforer; löst kisel, järn och aluminium; utbytbart och amorft järn och aluminium. För att följa eventuella förändringar i extrakten så analyserades den mikrobiella aktiviteten samt antal kultiverbara bakterier innan och efter experimentet. Som ett index för den mikrobiella aktiviteten så analyserades även jordrespirationen (CO_2-produktionen) en gång i veckan under experimentets gång. Ett försök gjordes även att karaktärisera de mikrobiella samhällena med hjälp av så kallad community level physiological profiling. Vid jämförelse av de fyra extraktens påverkan på jorden så indikerar resultaten från experimentet att de olika extraktens inverkan är väldigt lika gällande flera parameterar och att få kopplingar kan påvisas mellan dessa och den mikrobiella aktiviteten. Resultaten tyder på att vid en omblandning av jorden, till exempel efter en rotvälta, så kommer den påföljande omblandningen av de mikrobiella samhällena inte leda till någon signifikant direkt påverkan på jordens kemiska egenskaper samt cyklingen av näringsämnen.

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Abstract

Microorganisms are ubiquitous in soil and by influencing their surroundings; they act as important mediators in the cycling of nutrients, thereby indirectly affecting plant health. To gain more knowledge regarding how microorganisms affect their environment, a three month incubation was performed in which the microbial communities originating from the O-, E-, B- and C-horizon of a podzolic soil profile was extracted and re-inoculated into sterilized soil from each horizon. This was done to test how the microbial communities would influence the different horizons and to determine if they would affect the chemical soil properties differently. The experiment was also designed to assess the relation between the microbial activity and the chemical soil properties. To test the effect that the four extracts may have on soil, the following parameters were analyzed before and after the experiment: pH, total dissolved organic carbon, total dissolved nitrogen and phosphorus; dissolved low molecular mass organic acids and siderophores; dissolved silicon, iron and aluminum; exchangeable and amorphous iron and aluminum. To monitor possible changes in the different extracts, the experiment also included before and after analysis of microbial activity and number of cultivable bacteria. Soil respiration (CO_2 production) was further analyzed once a week during the incubation as it represents an index of microbial activity. Characterizations of the four microbial communities were also attempted before and after the experiment by community level physiological profiling. Comparing the influence that the four extracts have on soil, the results from the experiment indicate that the four extracts have very similar effect on many of the analyzed chemical parameters and that there are few connections between the chemical soil properties and microbial activity. The results indicate that upon mixing of soil, for example following a windthrow, the redistribution of the microbial communities will not have a significant direct effect on the chemical soil properties and cycling of nutrients.

1. Introduction

Soil microorganisms play a key role in many ecosystem processes. They have been demonstrated to be important in the aggregation of particles and in formation of soil (Chotte, 2005), in the dissolution of minerals (van Hees et al., 2004), in determining the diversity-productivity relationship of plants (Schnitzer et al., 2011), in determining the phosphorous (P) availability in mature ecosystems (Turner et al., 2012), but also to be important mediators in the cycling of sulfur (S), nitrogen (N), and carbon (C) (Kowalchuk and Stephen, 2001; Kertesz and Mirleau, 2004; Kandeler et al., 2005). In only one gram of soil, up to 10 billion microorganisms can be found, stemming from thousands of different species (Roselló-Mora and Amann, 2001). This means that the mixing of just one kilo gram of soil may affect the microenvironment of 10 trillion soil microorganisms.

In the Swedish forestry sector, severe storm damage have been a recurring problem ever since the beginning of the 19th century and previous studies have reported an increase in the volume of damage during the this period (Nilsson et al., 2004). One of the natural disturbances in forests that constitute a part of the storm damage is windthrows, which result in the uprooting of trees and redistribution of mineral soil (Jonsson and Dynesius, 1993). Disturbances like these have been demonstrated to be important factors in the generation of new microsites. These in turn act as seed beds for plants and associated ectomycorrhizal fungi, which will affect the succession and help to maintain the continuity of boreal forest ecosystems (Tedersoo et al., 2008). But what happens to the microbial community and its impact on the soil and its chemical properties when the soil becomes mixed?

Several studies have been conducted on the weathering rates induced by both sterile and non-sterile mor (O-horizon) solutions on mineral soil and feldspars; Raulund-Rasmussen et al. (1998) found that sterile natural organic solutes were more important weathering agents at high pH compared to conditions of low pH and that these solutes were more important in the weathering of mafic minerals (Ca, Fe, Mg) than in the weathering of felsic minerals (K-Na-feldspars); Lundström and Öhman (1990) and Lundström (1994) found that when subjecting natural silt, ground feldspars and soil to sterile and non-sterile mor extracts, weathering decreased in the presence of microorganisms as a consequence of consumption of organic acids. In contrast, a more recent study found that a higher bacterial production of low molecular mass organic acids (LMMOAs) induced a higher mobilization of iron (Fe) from phyllosilicates when bacterial communities from the different horizons of leptic podzols were added to the mineral (Balland-Bolou-Bi and Poswa, 2012). In the same study, it was also found that when the bacterial communities originated from soils with different chemical properties, they exhibited different abilities to mobilize Fe from the phyllosilicate. This was explained by the different abilities of the bacterial extracts to produce LMMOAs. Until now, few studies have been conducted in which natural microbial communities have been re-inoculated into their natural habitat with the purpose to investigate its effect on chemical soil properties.

Given the importance of microbial communities in nutrient availability and plant health (Clivot et al., 2012) it is of high value to develop our understanding of how the microbial

communities influence the soil, and more importantly, what happens as the soil community becomes redistributed? Will the community keep their originate functions and influence the chemistry in a different way or will it adapt its function to the new surrounding? Investigations like these can give us indications on of how the microbial community responds to natural disturbances and if and how this could lead to a change in the chemical properties and cycling of nutrients in soil.

1.1 Aim and hypotheses

The aim of this study is to increase the knowledge of how the microbial communities of podzolic soil affect the chemical properties in their surrounding at a sudden change in environment and to determine if the microbial communities originating from different podzolic soil horizons will have different influence on the chemical soil properties i.e. will a change in microbial community be reflected in the chemical properties of the soil? A second aim is to determine if there is a connection present between microbial activity and the chemical soil properties. The hypotheses are that 1) the chemical soil properties of a podzolic soil horizon will undergo a change as the microbial community of the horizon changes and that 2) changes in nutrient availability and metal mobilization in a podzolic soil profile can be related to the microbial activity.

To address these hypotheses, an experiment was conducted where the microbial communities inhabiting the four distinct horizons of a podzolic soil (O, E, B, C) was extracted and re-inoculated into sterilized soil from each horizon. This was done to monitor the influence that the four extracts would have on the soil and to see if they would have a different effect on soil properties.

1.2 Dynamics of nutrients: weathering mechanisms and distribution of elements in soil

1.2.1 Chemical weathering

Soils consist of a number of crystalline and amorphous mineral phases created during mechanical and chemical weathering of the crust and it is upon accumulation of these weathering products that thick soils develop (Schlesinger, 1997; van Breemen and Buurman, 2002). With its close link to soil processes, chemical weathering of soil particles leads to a transformation of the material under the influence of water, organic and inorganic acids, complexing agents and oxygen (Rode, 1070; Schlesinger, 1997; van Breemen and Buurman, 2002).

One of the most basic weathering processes is *hydration* in which water molecules are incorporated into the crystal lattice, leading to expansion and mechanical deformation of the mineral. In *oxidation* and *reduction* reactions, oxygen is either added or removed from compounds at the mineral surface (e.g. in oxidation of FeO to Fe_2O_3) removing Fe oxides

from solution or precipitating new particles (Nagle 2000; Schaetzl and Anderson, 2005). The most important weathering process for silicates is *hydrolysis* in which a water molecule is split into a proton (H^+) and a hydroxyl ion (OH^-). Protons in turn will contribute to a lowering of pH but also perform and exchange with cations at mineral surfaces. As protons further dislodge metal ions from minerals, ions will subsequently become free to react with the free hydroxyl ions, forming new substances (e.g. clays). Free protons can further be added to soil solution by dissociation of acids which results in an increase of hydrolysis at low pH (Schaetzl and Anderson, 2005). The main process of chemical weathering in soil is the *carbonation reaction* in which CO_2 reacts with H_2O, forming carbonic acid (H_2CO_3) which attacks minerals leading to dissolution:

$$H_2O + CO_2 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H_2CO_3$$

By respiration, microbes and plant roots deliver CO_2 to soil solution through which the acid component can travel to substantial depth down the soil profile (Schlesinger, 1997). The significance of the carbonation reaction however decreases with decreasing pH (significant at pH >8.5). This leads to the fact that in northern soils where topsoil exhibit low pH, the carbonation reaction is mainly present in the lower horizons. In these areas, the absence of carbonic acid in top soil is attributed to the greater presence of high molecular and low molecular mass organic acids. Due to a colder climate and slower degradation, these are allowed to accumulate to a greater degree (Johnson et al., 1977). Organic acids such as fulvic, humic and phenolic acids are added to soil during microbial degradation of organic material. In addition to contributing to total acidity in soil, organic acids also contribute to mineral weathering by chelating metals ions (e.g. Fe, Al) which lowers the concentration of inorganic metals in soil solution, leading to disequilibrium and continued weathering (Schlesinger, 1997).

1.2.2 Biological weathering

All living organisms on earth have a need for mineral nutrients (e.g. Fe) for which the lithosphere is the primary source. By affecting its surroundings (e.g. during pedogenesis), the biological and microbial activity in soil will help to make these nutrients more accessible (Sokolova, 2011), inducing weathering rates of rocks and minerals orders of magnitude faster than in abiotic systems (Dong, 2010). In soil, plants and microbial communities contribute to weathering both by mechanical and chemical actions. In 2009, Calvaruso et al. (2009) showed that the dissolution of clay-sized soil minerals was higher in the rhizosphere of Norway spruce and oak compared to surrounding bulk soil, something which was attributed to high root production of protons. It has also been shown that weathering may vary between different tree species on which Augusto et al. (2000) reported that Norway spruce and Scots pine induced notably higher dissolution rates than broadleaved tree species did. In addition to protons, plant roots also excrete carbohydrates produced during photosynthesis at their root tips, attracting several different fungal species. The fungi will in turn redistribute inorganic nutrients (e.g. K, Mg, Ca, N, P and Fe) back to the host plant through its mycelia this was creating a symbiosis between tree and fungi.

In boreal forests, >95% of tree root tips are covered by ectomycorrhizal fungal mantles (Näsholm et al., 2013). This symbiosis between tree and fungi results in enhanced weathering as demonstrated by Ochs et al. (1993) who reported that exudates from ectomycorrhizal trees significantly increased the weathering of aluminum oxides (Υ-Al_2O_3) in contrast to non-ectomycorrhizal tree exudates. In a more recent study, Dickie et al. (2002) found that infection of Northern oak tree seedlings with ectomycorrhizal fungi increased N uptake compared to non-infected seedlings. Other benefits which have been found in the symbiosis between plant roots and mycorrhizal fungi are increased plant stress tolerance to factors such as drought, temperature decrease and decreasing pH; plant roots in association with fungi have also demonstrated a higher tolerance to soil-borne diseases (Lanthier, 2009). As fungi and tree roots form this symbiosis, the physicochemical and biological conditions in the surrounding soil will change, creating the so called ectomycorrhizosphere. The special conditions of this part of the soil column will in turn help select the microbial communities present (Calvaruso et al., 2007), a process called the "mycorrhizosphere effect" (Linderman, 1988). The mycorrhisosphere further seems to attract microorganisms effective in solubilizing Fe and P bearing minerals. On that subject Calvaruso et al. (2007) found that bacterial isolates from the soil-fungal interface of an oak tree stand could mobilize Fe and P compared to isolates from bulk soil of which the majority could not.

1.2.3 Microbial adhesion to mineral surfaces and production of biofilms

In more detail, there are several means by which microorganisms can solubilize minerals and keep metals in solution; for example by coating mineral surfaces with polymers or by direct attachment to mineral surfaces (Bennett et al., 2001). During adhesion of microbial cells to a mineral site, microorganisms exhibit a direct effect on mineral dissolution. According to Sokolova (2011) as much as 80-90 % of microbial cells in soil adhere to the surface of solid particles, presumably by the forces of van der Waal, hydrophobic and electrostatic interactions. Attached at the surface; microorganisms act as the cathodic part of the electrolyte, acquiring energy by transporting electrons from the mineral to the cell via electrically conductive pili (Gorby et al., 2006). Another way by which bacteria contribute to the weathering of minerals is by coating mineral surfaces with an extracellular polymeric substance (EPS). Through its ability to retain water, the EPS is known to increase mineral fracturing and hydrolysis (Konhauser, 2006). During substrate shortage, the substance can also work as a C and energy source for bacteria (Sheng et al., 2010) promoting the exudation of LMMOAs. The EPS can further destabilize minerals as it contains carboxyl groups which can dissociate into protons and acids. Acting as a nucleation site it can also be involved in the formation of authigenic minerals (Bennett et al., 2001). As bacteria, fungal species are also known to colonize minerals by attaching their mycelia to its surface, this again leads to increased retention time of water. It also promotes the formation of microcavities which in turn result in an increased surface area susceptible to weathering (Bonneville et al., 2009).

1.2.4 Microbial weathering by low molecular mass organic acids

Production and excretion of LMMOAs is another strategy by which the microbial community can solubilize minerals in their surroundings. LMMOAs are organic compounds which depending on the number of carboxylic groups are monofunctional, bidentate or tridentate. The greater part of LMMOAs of fungi and bacteria are produced as a byproduct of fermentation and in intermediate steps of aerobic respiration of glucose (Konhauser, 2006). There are several ways by which LMMOAs contribute to the dissolution of minerals; when released into the surrounding of its producer, the majority of LMMOAs dissociate into protons and organic anions. The release of protons increases the proton-promoted dissolution by which protons attack metal ligands at the mineral surface, leading to a weakening of the metal-oxygen bond and a subsequent release of a metal cation. Organic anions further induce dissolution by reacting with metal cations at mineral surfaces. This leads to further instability in the metal-oxygen bonds as anions form metal-chelate complexes which further detach cations from the mineral surface. Anions will also form complexes with metal cations in solution, which leads to a lowering of the saturation state of the solution, promoting further dissolution (Konhauser, 2006). Complexation is also affected by the concentration and type of metal in question but also by the pH of soil solution (fig 1).

In the aspect of natural conditions, concentrations of LMMOAs found in soil are relatively low and range between 1 to 50 µM (Jones et al., 2003). Earlier studies on the presence of LMMOAs in podzol soils have reported findings of several different types of LMMOAs including citric, isocitric, oxalic, acetic, formic, shikimic, butyric and malic acid. The pattern for distribution was higher concentrations in the organic O-horizon followed by a gradual decline with depth (van Hees et al., 2000; Holmström et al., 2003; Ali et al., 2011).

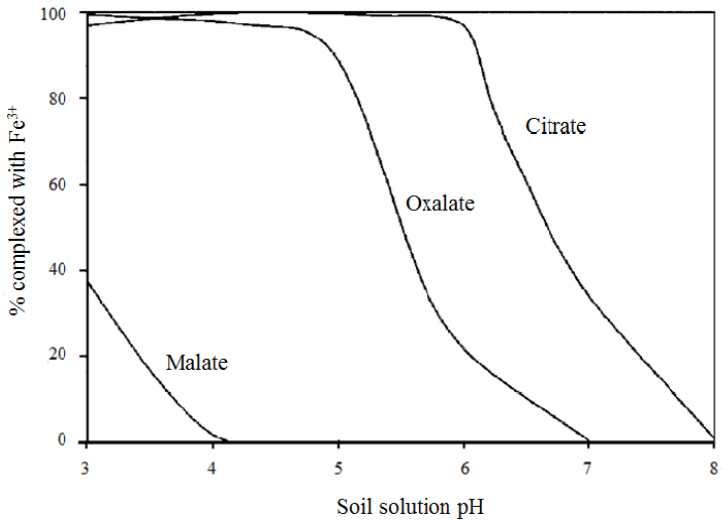

Figure 1. The graph demonstrates how complexation between metal ions and LMMOAs is affected by different soil solution pH (Jones, 1998).

1.2.5 Microbial weathering by siderophores

For almost all microorganisms, Fe is an essential element required for numerous physiological functions (e.g. in the production of Fe cofactors, cytochromes, hydroperoxidase, ribonucleotide and nitrogenase). However, because of low solubility of Fe oxyhydroxides under aerobic conditions and neutral pH, Fe is often highly unavailable and only present in concentrations up to 10^{-17} M. Nevertheless, the majority of microorganisms require concentrations up to 10^{-6} M to support growth (Hersman et al., 2000), which leads to the demand for an effective Fe acquisition system. For several bacteria, fungi and plants, the answer to this problem is to produce siderophores, another kind of multidentate organic molecule which often is exuded in combination with LMMOAs (Holmström et al., 2004; Essén, 2007; Reichard et al., 2007). In addition to promoting dissolution of minerals, production of these ligands can further favor microorganisms in the competition for dissolved Fe at low pH. Compared to LMMOAs, siderophores have a higher formation constant for Fe (e.g. log $K_{f\ (siderophore)} = 10^{25\text{-}50}$, log $K_{f\ (oxalic\ acid)} = 10^{8}$) which makes them even more preferential under Fe limited conditions.

The steps leading to the acquisition of metal ions by siderophores begins with an intracellular production of the ligand, followed by exudation from the microbial cell. The ligand will then form a complex with a metal ion (e.g. Fe^{3+}) either from solution or by destabilization of a mineral surface. Back at the cell, the metal-siderophore complex can either be assimilated by the organism when recognized at a receptor, or be reduced at the cell surface upon which the metal ion itself is assimilated (Kraemer, 2004).

When released into the surrounding of its producer, siderophores affect mineral dissolution in three ways; firstly by forming complexes with metal ions at mineral surfaces (fig 2) induced by release of a proton, secondly by proton-promoted dissolution and thirdly by complexing with metals in solution which leads to a lowering the saturation state. In addition to Fe, siderophores are also known to form complexes with other metals such as Mn(III) (Geszvain et al., 2012) due to their ionic potential which is similar to that of Fe(III) (Konhauser, 2006). Previous studies have reported the occurrence of nM range concentrations of fungal hydroxamate siderophores ferrichrome and ferricrocin in podzol soil with the general pattern of higher concentrations in the top (O) horizon followed by lower concentrations in the eluvial (E) horizon, (Holmström et al., 2004; Essén et al., 2006; Ali et al., 2011).

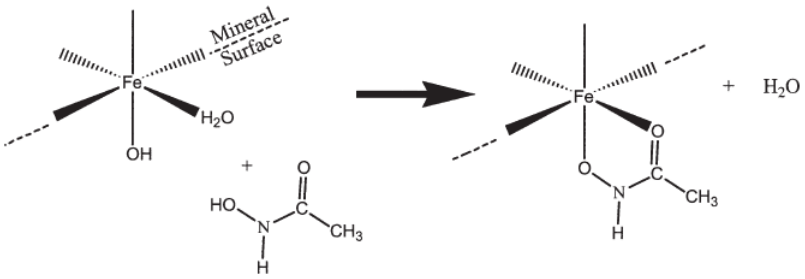

Figure 2. Adsorption of the siderophore-like ligand acetohydroxamic acid to an Fe oxide surface resulting in the formation of an inner sphere surface complex (Kraemer, 2004 after Holmen and Casey, 1996).

1.2.6 Silicon, aluminum and iron in soil

Besides oxygen (O), silicon (Si), aluminum (Al) and Fe are the most abundant elements in soil and constitutes in average 27%, 4% and 7% of soil mass (Ma, 2005). Together, these three elements represent important indicators of mineral weathering (van Hees et al., 2004). Considering the relation between Al, Fe and Si, the ratio of Si to sesquioxides (Fe and Al) represents a comparative index of soil formation and the degree of podzolization, during which the ratio resumes at high values due to the immobility of Si compared to that of Fe and Al (Schlesinger, 1997).

Although Si is not considered an essential nutrient, it is regarded as a beneficial nutrient, decreasing Mn-toxicity in plants and increasing its resistance to multiple stress (Rogalla and Römheld, 2002; Ma, 2005). Since the atmospheric addition of Si to soil is very small and the main source of the element comes from mineral dissolution, Si is a good indicator of mineral weathering (Schlesinger, 1997). The concentration of dissolved Si in soil is controlled by hydrolysis and dissolution of (1) primary silicates, (2) secondary minerals containing silica and (3) phytoliths (Watteau and Villemin, 2001). It is the weathering of primary silicates which contributes to the fertility and electrolyte content among which Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Mn^{2+} and Fe^{2+} are major decomposition products (Sposito, 2008). Typically, the reservoir of Si liable to weathering is larger under deciduous forest than under coniferous forest, which is coupled to the reservoir in above-ground biomass (Bartoli, 1983).

Al is the third most abundant element in the earth´s crust and in most soils it resides as oxides and aluminosilicates, forms in which the element does not pose a threat to the plant and microbial community. In acid soil however, part of the Al will be present in solubilized form as Al^{3+} during which it is considered to be a toxin to both plants and microorganisms. Therefor many organisms respond to Al-stress by secreting LMMOAs which complex strongly with the metal ions (i.e. Al^{3+}), preventing them from binding to cellular components (Kawai et al., 2000; Ma, 2004). On this matter it has been appreciated that about 30 % of Al in solution in podzolic O-horizons are bound to these organic solutes (van Hees et al., 1996; Holmström et al., 2004). The solubility and speciation of Al is further very pH dependent where the fraction of Al that is present in inorganic form will increase with a lowering of soil pH (fig 3) (Lundström and Giesler, 1995).

Fe is the fourth most abundant element in the earth´s crust and in opposite to Al, Fe is considered an important essential micronutrient. Under aerobic conditions microorganisms, among many crucial purposes, depend on the element (Fe^{2+}) for reduction of oxygen for synthesis of ATP, reduction of ribotide precursors of DNA and for production of heme (Neilands, 1995). Under anaerobic conditions, Fe^{3+} is in opposite used as an electron acceptor, for example in the metabolism of acetate (Lovely and Phillips, 1986). The main source of Fe for the microbial and plant communities in soil are the Fe(III) oxides which are formed from the weathering of primary minerals (Schwertmann, 1991). The most stable hence the most common Fe oxides in soil are goethite and hematite (Chesworth, 2008). The availability of Fe in soil is regulated by the solubility of Fe oxides and by the rate of dissolution (mechanisms described in section 1.1) and as for Al the solubility and speciation of the element is affected

by pH and redox potential (fig 3). The dissolution of Fe-bearing minerals hematite and goethite will further be affected by the substitution of Fe-for-Al where an increased incorporation of Al will result in a higher stability of the mineral (Torrent et al., 1987).

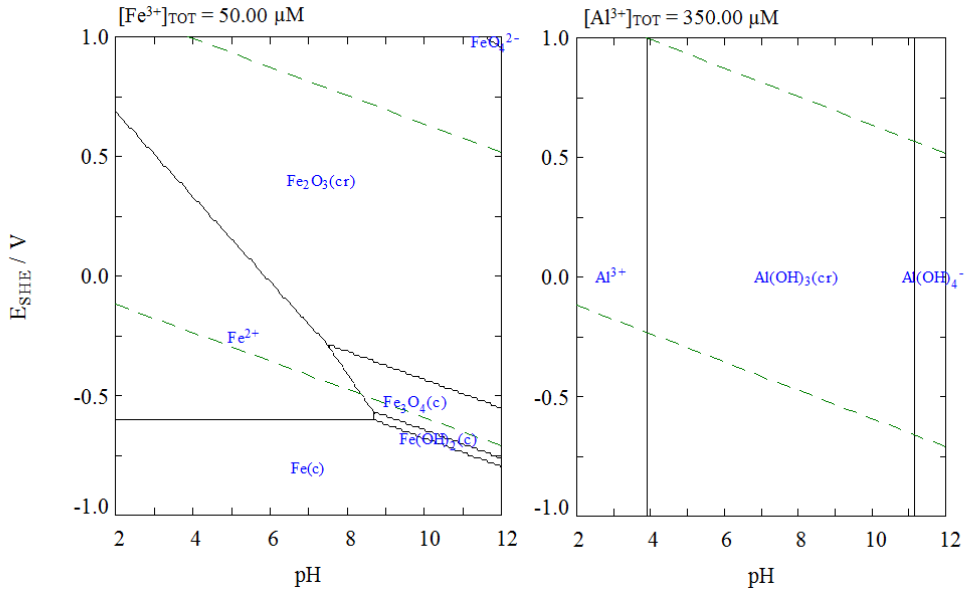

Figure 3. Predominance diagram for Fe and Al. Diagrams are constructed for the average concentration found in the O and E horizon at Norunda from the present investigation, using Medusa software.

1.2.7 Microbial interactions with carbon, nitrogen and phosphorus in soil

In soil, the microbial communities play an important role in the biogeochemical transformation of organic matter, which in turn affects soil fertility. The microbes has a capacity to work both as a sink and a source for nutrients such as C, N and P by both consuming but also releasing nutrients bound in organic and mineral complexes (Aponte et al., 2010). Immobilization, i.e. microbial conversion of inorganic nutrients into organic nutrients predominates in the surface layer of soil. In the deeper profile, the opposite mechanism, mineralization is the primary process (Schlesinger, 1997).

According to Kandeler et al. (2005) changes in soil organic C dynamics are intimately connected to or even driven by changes in microbial activities. In principal, organic C is delivered to soil as dead plant and microbial residues together with plant and microbial exudates (e.g. LMMOAs), which in turn constitutes the most important energy sources for soil microorganisms. During degradation, part of the organic C will undergo humification thereby delivering a more complex form of organic debris (e.g. fulvic acids, humic acids and humin), which in turn will affect the surrounding soil properties, for example by increasing soil aggregation and stability and by chelating metal cations from solution (Bot and Benites, 2005; Kandeler et al., 2005). Through microbial respiration and fermentation, other parts of organic debris will be mineralized creating the end product of CO_2 and the redistribution of inorganic nutrients such as N and P into the surroundings (Konhauser, 2006).

The primary reserve of P is located in rocks. In acid soil, mineral P is mostly present in associations with hydrated oxides of Fe, Al and Mn. The element is also present in primary minerals such as apatite, hydroxyapatite and oxyapatite of which one of the main characteristics are their low solubility (Rodríguez and Fraga, 1999). The microbial community will be involved in the regulation of P through weathering and precipitation of P-containing minerals and through mineralization and immobilization of P-compounds.
Regarding weathering, previous studies have determined the ability of both fungi and bacteria to solubilize P minerals (Nahas, 1996) and according to Rodríguez and Fraga (1999) the principal mechanism for solubilization of P is considered to be LMMOAs synthesized by microorganisms. By degradation of organic material and production of LMMOAs, microorganisms will also indirectly contribute to the precipitation of P by lowering of soil pH. In acid soils (low pH), inorganic P reacts with dissolved Fe and Al which subsequently can lead to a precipitation of P in phosphate minerals (Hyland et al., 2005). In the aspect of mineralization, microbes will aid the plant community by solubilizing organic P and making it available for the plant in the form of orthophosphate (PO_4^{3-}). In addition, the microbial consumption of orthophosphates will turn the element into its organic form again which will only be made available to plants upon death and re-degradation of the microbial cells (Rodríguez and Fraga, 1999; Hyland et al., 2005).

Together with C, O and H, N is one of the main constituents of the organic compounds that together form an organism (Gottchalk, 1986). As for P, the soil microbial community is involved in the regulation of N through the processes of mineralization and immobilization but also through nitrification and denitrification. In mineralization, the microbes decompose organic N to ammonium (NH_4^+) via ammonia (NH_3), making the nutrient available to plants. In the next step, microbes oxidize parts of the ammonium to nitrate (NO_3^-) as a means to gain energy, leading to increased availability of N for plants. During denitrification, NO_3^- is conversely made unavailable by transformation into N_2 (passing the steps of NO_2^-, NO, N_2O) as microorganisms use the molecule as an oxygen source (Johnson et al., 2005). The NO_3^- can also be immobilized by microbial uptake upon which the NO_3^- is reduced back to NH_4^+ by NO_3^- reductase, stimulating microbial growth (Schlesinger, 1997).

1.3 Podzolic soil

Podzols can be found all over the world but are most extensively distributed in Scandinavia, northwest of Russia and Canada. The soil type covers approximately 485 million ha of land worldwide (fig 4); in Sweden it covers 75 % of the total land area (Melkerud et al., 2000).
Podzols are mineral soils, which are characterized by a dark surface O-horizon rich in organic material. Next comes the eluviated E-horizon which contains less amounts of base cations, Al and Fe compared to the O-horizon and is enriched in minerals resistant to weathering. Due to high leaching and formation of metal-organic complexes, this horizon is characterized by its grey appearance. Below the E-horizon comes the illuvial B-horizon. This horizon is rich in precipitated Al, Fe and residual Si which gives the soil its typical brown-orange-reddish color.

The B-horizon is in turn underlain by the C-horizon which remains relatively unaffected, showing few signs of soil formation (Lundström et al., 2000a). Podzols appear in a variety of climates worldwide but are most common under cool humid conditions. The soil type is dominant in humid boreal forest zones, where it mainly forms on glacial, mixed parent material often rich in weatherable minerals (Lundström et al., 2000b; van Breemen and Buurman, 2002).

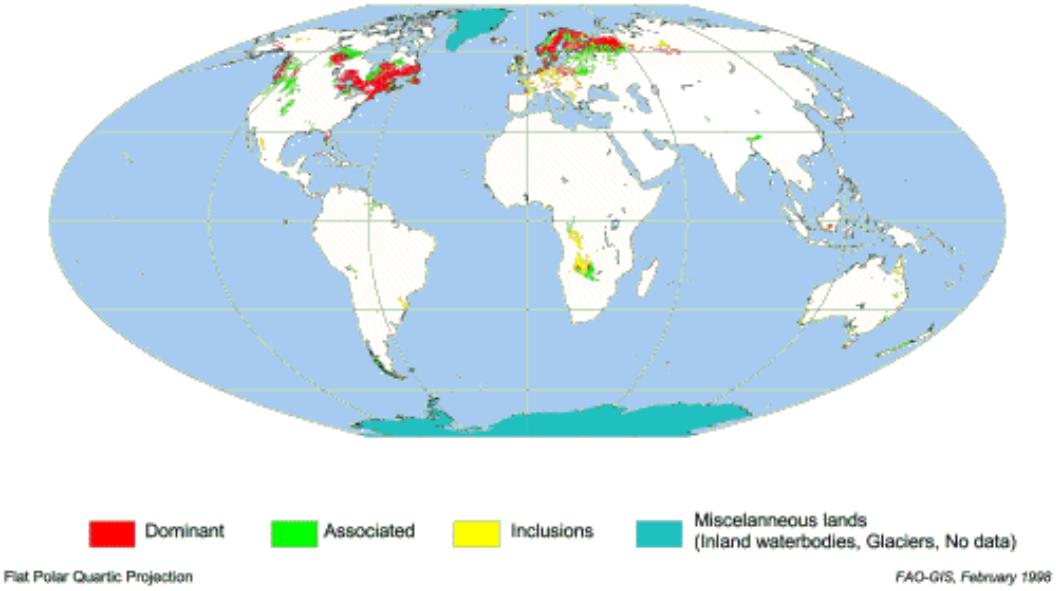

Figure 4. A map showing the worldwide distribution of podzols (UNCS).

1.3.1 Podzolization and distribution of Fe, Al and Si through podzolic soil profiles

During podzolization, acidic soil profiles undergo a gradual change where the horizons slowly transform into the distinct layers of a podzol. Depending on climatic factors such as precipitation and temperature, the formation time for podzols range between 350 and 1000 years (Lundström et al., 2000a). Podzolization is especially intense in coniferous forests situated in cold climates where the coniferous trees contribute to litterfall rich in phenolic compounds and LMMOAs, which degrade slowly due to cool conditions (Schlesinger, 1997). When podzolization occurs, (1) organic compounds (e.g. LMMOAs) together with base cations (e.g. Fe^{3+}, Al^{3+}, Si^{4+}) are leached from the surface layer of soil (2) becomes transported down the soil column (3) and are subsequently precipitated in deeper horizons. To date, several different theories have been formulated to explain podzolization (e.g. *the fulvate theory*, Petersen 1976, *the allophane theory*, Anderson et al., 1982, *the low molecular weight acids theory*, Lundström et al., 1995) where the current view explains the processes as a combination of the fulvate theory and the low molecular weight acids theory (van Breemen and Buurman, 2002). This combination of theories suggest that unsaturated high molecular mass organic acids (HMMOAs) and LMMOAs produced in the top layers (O-horizon) form complexes with Al and Fe ions leading to the dissolution of primary and secondary minerals (fig 5). In the example below, oxalic acid works to dissolve albite. As the dissolution

proceeds, oxalic acid chelates Al^{3+} forming an organo-metallic complex and at the same time Na^{+} and Si^{4+} is released into solution. These weathering processes further release nutrient cations such as K^{+}, Mg^{2+} and Ca^{2+} which support as a resupply during leaching losses (Giesler et al., 2000).

$$2H_2C_2O_4 + 4H_2O + NaAlSi_3O_8 \rightarrow Al(C_2O_4)^{+} + Na^{+} + C_2O_4^{2-} + 3H_4SiO_4$$

oxalic acid — albite — Al-oxalate — oxalate ion — silica (aq)

As the water soluble organo-metallic complexes percolate down the soil column, an eluviated E-horizon is formed, enriched in resistant minerals such as quartz. As the complexes continue their downward migration, the microbial consumption of LMMOAs parallel to the saturation of HMM organic complexes and an increasing pH will lead to a subsequent precipitation of Fe and Al oxides (e.g. ferrihydrite, allophane, imogolite and goethite) forming the B-horizon.

The processes involved in podzolization will consequently affect the distribution and form of Fe, Al and Si present at different depth in the soil profile. During natural conditions, the amount of the dissolved elements tends to decrease with depth, this agrees with the fact that the amount of Fe and Al bound to LMMOAs also decreases with depth (van Hees et al., 2000). A previous study by Giesler et al. (2000) found that the greatest concentration of total soluble Fe and Al were present in the O- and E-horizon and decreased in the B-horizon whereas Si had its maximum in the E-horizon. Riise et al. (2000) further showed that the concentration of Al was four to five times higher than that of Fe and that both Fe and Al were present mostly in the colloidal form in all horizons whereas Si was present in dissolved form (H_4SiO_4). The highest amount of (oxalate extractable) amorphous Al and Fe is normally found in the B-horizon (Degórski, 2007) whereas the amount of exchangeable cations decrease with horizon (Nissinen et al., 1998).

1.3.2 Spatial distribution of microbial communities with different soil characteristics

The combinations of acidic parent material, input of coniferous plant litter and slow degradation of organic acids results in the acidic environment of podzolic soils, where pH normally ranges from 3 to 5.5. Acidic soils are in turn less functional for bacteria and actinomycetes, which lead to a dominance of fungi in the microbial community (Giri et al., 2005). In 2001, Ekelund et al. (2001) investigated the numbers and biomass of protozoa, bacteria and fungi at various soil depths at one dry beech forest, one peaty spruce forest and one dry spruce forest. They found that within the top 20 cm, the biomass of fungi was 20, 8 and 60 orders of magnitude higher, respectively, than that of bacteria. At the peaty spruce forest, they also found that bacterial biomass showed a sudden increase at depth (42 cm) compared to that of surface conditions (5 cm), explained as a result of lower presence of protozoa which control bacterial populations. Aside from this finding they concluded that the microbial biomass had a general tendency to decrease with depth, something also concluded by Fritze et al. (2000) who studied the distribution of biomass in nine different podzols.

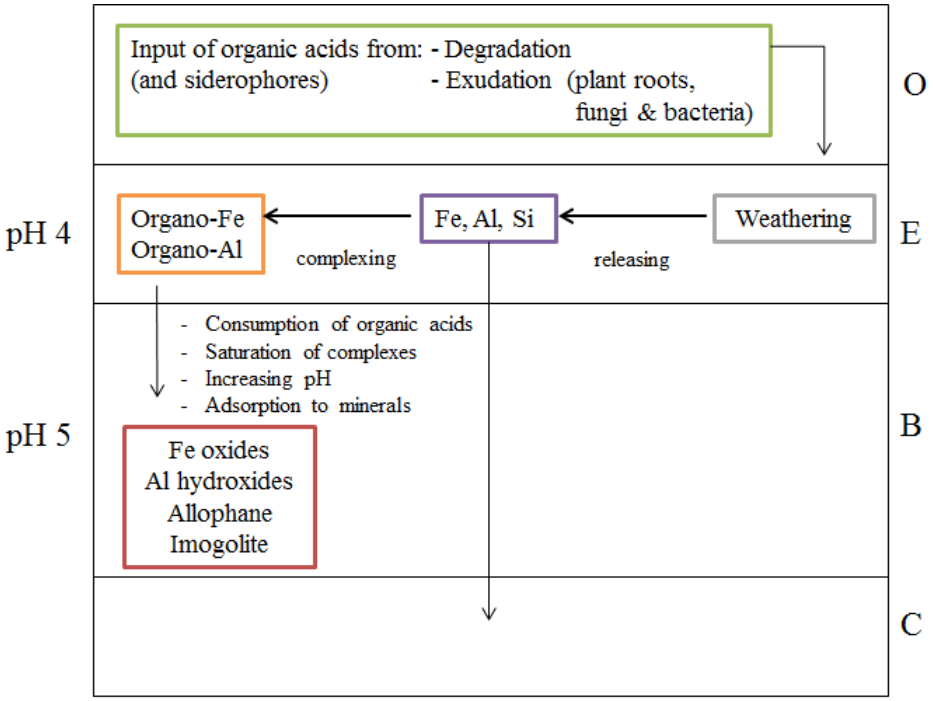

Figure 5. A visualization of the podzolization process. Input of organic acids from the degradation of organic material and exudation of siderophores and LMMOAs by microorganisms in the O-horizon results in the weathering of minerals in the top soil. As dissolved elements and organo-metallic complexes percolates down ward, the eluviated E-horizon starts to form. During the organo-metallic transport, precipitation will occur as a consequence of increasing pH, saturation of complexes and consumption of LMMOAs, leading to the formation of the B-horizon. At the bottom of the profile lies the relatively unaffected C-horizon. Modified from Gustafsson et al., (1998).

In accordance with Ekelund et al. (2001), the results from a similar study conducted on podzols by Nikinov et al. (2001) showed that fungi constituted 90 % of the total biomass in soil. They also found that the microbial population of a spruce forest podzol mainly were concentrated within the surface layer (10-15 cm), but also that there was no drastic decrease present in microbial abundance and biomass until beneath the illuvial horizon (B-horizon).

In addition to pH, there are several other parameters that vary with soil depth, which also may influence the spatial distribution and community structure of microbes; soil organic matter (SOM) and the quality of C substrates tend to decrease with depth, something which will be reflected in the composition of the microbial community. This decline is explained by the input of root exudates, surface litter and root detritus in top soil and also by the slower rate of C input to lower horizons (Fierer et al., 2003). Also macro nutrients such as N, P, Na, K, Ca and Mg have previously been demonstrated to decrease with soil depth (Gupta and Rorison, 1975). Soil moisture will also vary through the profile and its variations have previously been strongly correlated to respiration and microbial biomass and shown to affect bacterial and fungal composition (Schimel et al., 1999; Fierer et al., 2003). Drought stress can further lead to a 30% decrease in C resources as these become bound in cytoplasmic osmotic protection molecules, reducing microbial activity and population growth (Schimel et al., 1989).

2. Material and methods

2.1 Field area and sampling

Figure 6. The organic rich O-horizon (0-6 cm), the thin eluviated E-horizon (6-10 cm), the clayey B-horizon (10-50 cm) and the sandy pebble rich C-horizon (+50 cm).

Experiments were conducted on soil collected from the site of Norunda research station (60°05'N; 17°29'E) situated 30 km outside of Uppsala in the southern part of the boreal forest zone (fig 7). The location lies 45 m above sea level and experiences a mean annual rainfall of 527 mm and a mean annual temperature of 5.5°C (1961-1990) (Lundin et al., 1999). The research station, which is included in ICOS-Sweden and operated by Lund University, was established in 1994. At the sight, parameters like soil humidity, soil temperature, soil respiration and production of CH_4 is continuously monitored all year around. The area is described as a mature boreal forest which is dominated by Norway spruce *Picea abies* and Scots pine (*Pinus sylvestris*). The site also has some features of deciduous trees, most commonly birch (*Betula sp.*) together with a ground vegetation mainly consisting of mosses and sporadic stands of dwarf shrubs. Growing season, with a threshold of 5°C usually lasts from mid-April until the second half of October. The soils in the area are characterized as podzolised dystric regosols (Lindroth et al., 1998; ICOS-Sweden, 2011).

All soil for the experiments was collected under spruce in the beginning of August 2012, approximately 2 kg from each horizon (O, E, B, and C) (fig 6). Fresh soil intended for microbial extraction and analysis was put into sterile tubes and kept in a coolbox and later stored at 4°C until analysis. The remaining soil was left to air-dry on sterile petri dishes for approximately three weeks before sample preparation and analysis. After drying, the soil was sieved with a 4 mm mesh and cleared from the largest fraction roots, needles and moss.

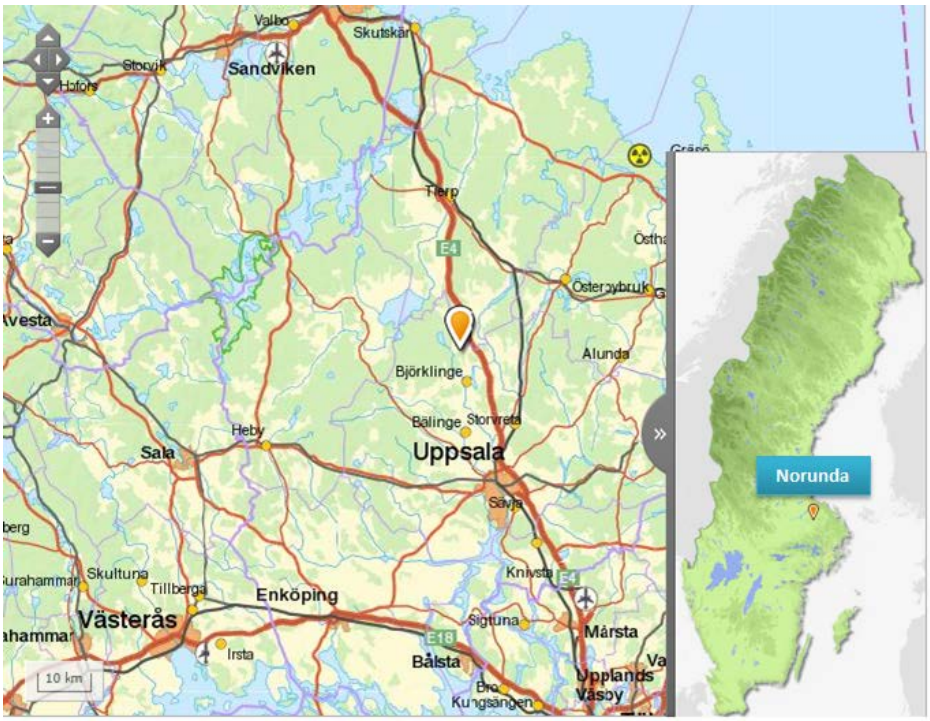

Figure 7. The sampling site Norunda Research station is located 80 km outside of Uppsala (Lantmäteriet).

2.2 Experimental setup

For the experiment, glass bottles with a volume of 120 mL were used to incubate soil from the O-, E-, B- and C-horizon (fig 8) of the podzolic soil from Norunda. For the E-, B- and C-horizon; 28 grams of air-dried and sieved soil were inserted into 15 bottles each, resulting in a total of 45 bottles for the three different horizons (see table 1). Due the lower density of the O-horizon (i.e. greater volume to weight ratio), each replicate was split into two by filling two paired bottles (e.g. O1A & O1B) with 14 grams of soil each, resulting in a total of 30 bottles for the O-horizon. Bottles were sealed with rubber stoppers and aluminum caps.

Figure 8. Incubation bottles with soil from each horizon.

2.2.1 Sterilization of soil

To be able to control the microbial population in the soil of each bottle, all soil was sterilized at The Royal Institute of Technology, KTH, using an electron beam. After distribution of soil and closing of bottles, sterilization was obtained by radiating each bottle, a method which has been previously tested on soil (McLaren et al., 1957). One of the advantages with using an electron beam is its high electron energy which results in effective sterilization. This high electron energy also reduces the exposition time of the material and allows for a large set of samples to be sterilized in a short amount of time (Silindir and Özer, 2009). Before the method was implemented, a test was performed by sterilizing soil from all four horizons. Soils were then extracted using the same procedure as in the experiment after which the extracts were used to inoculate agar plates. After four weeks in room temperature, no sign of microbial growth could be detected at visual control of the plates. To obtain a sterilization of the soil, each flask was radiated with energy of 7 MeV during 2x4 minutes, resulting in a dose of 50 kGy. To avoid heating, all bottles were radiated twice with a resting period of approximately 30 minutes in between, which allowed the material to cool down. This reduced the heating of the bottle to a maximum temperature of approximately 38°C.

2.2.2 Extraction of microorganisms from fresh soil

Because the purpose of this experiment was to investigate how a microbial community from a certain podzol horizon may behave when inserted into another soil horizon, all inoculations were prepared from fresh podzolic soil. For the extraction of microorganisms, 2 g of fresh soil was suspended in 20 ml sterilized Milli-Q water in 50 ml tubes with the addition of glass pearls to increase the separation between soil particles and microorganisms. Tubes were then shaken hard on a reciprocal shaker for 2 hours followed by softer shaking overnight. Before collection of fluid, tubes were left standing for 1 hour to allow bigger particles to settle. Extracts from each horizon were then transferred into sterile glass bottles and homogenized before immediate inoculation of soil.

2.2.3 Inoculation of sterilized soil

To start the experiment, the sterilized O-, E-, B- and C-horizon soil in the prepared glass bottles were inoculated with soil extracts. To be able to compare how a microbial community originating from a certain horizon would behave as the surrounding changes and how a change in microbial community would affect the soil properties, each soil horizon was inoculated with extract from each horizon. For the O-horizon, 3 pairs of bottles (named “A” and “B”, equaling 1 bottle for the other horizons) was either inoculated with O-horizon extract, E-horizon extract, B-horizon extract or C-horizon extract. For the E-, B- and C-horizon, 3 bottles from each horizon was inoculated with each extract (see table 1). Due to the fact that the O- and E-horizon of a podzol contains higher amounts of microorganisms compared to the B- and C-horizons, the inoculum volumes of O- and E-horizon extract used were set to 2 ml while the inoculum volumes of B- and C-horizon extract were set to 5 ml. To be able to separate microbial effects (i.e. biotic affects) from non-microbial processes (i.e. abiotic processes) in the soil, abiotic controls were prepared which functioned as control

experiments for abiotic processes. Instead of adding microbial extracts, these were "inoculated" with sterilized Milli-Q water.

Table 1. The following table lists the labeling of inoculation bottles, the sort of inocula that was inserted into what bottle and soil and the total number of bottles for each kind of inoculation.

Inocula	O horizon	E horizon	B horizon	C horizon
O extract	O1A-O3B	E1-E3	B1-B3	C1-C3
E extract	O4A-O6B	E4-E6	B4-B6	C4-C6
B extract	O7A-O9B	E7-E9	B7-B9	C7-C9
C extract	O10A-O12B	E10-E12	B10-B12	C10-C12
Sterile Milli-Q water (abiotic controls)	O13A-O15B	E13-E15	B13-B15	C13-C15
Total number of bottles	30	15	15	15

2.2.4 Experimental conditions of soil incubations

To avoid contamination of incubation bottles, these were kept inside a laminar flow hood during the full duration of the experiment (except at CO_2-analysis). The flow hood subjected the bottles to a comparatively constant temperature of 22°C (fig 9).To avoid the effects that an increasing pCO_2 could have on the soil, bottles were stoppered with foam rubber stoppers which allowed for circulation of air at the same time that it prevented evaporation. Rubber stoppers also functioned as an extra guard against contamination and were sterilized once a week. To maintain relatively constant soil moisture of 60-75 % water holding capacity, bottles were weighed and watered with sterilized Milli-Q water once every week. To reduce the effect that UV-light could have on soil and to mimic the dark conditions of a real conditions, bottles were wrapped in aluminum foil.

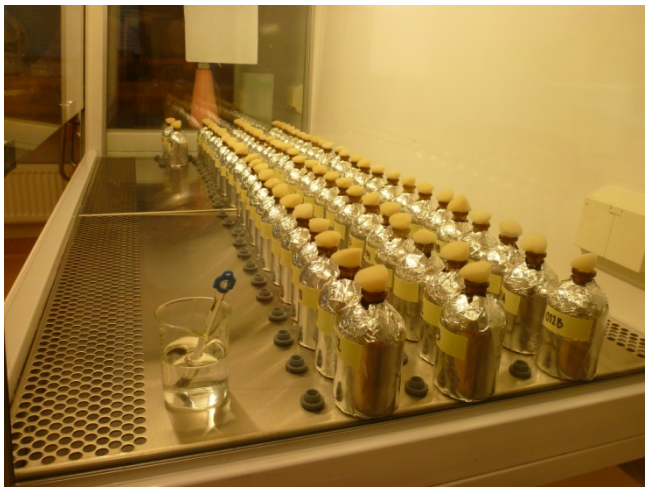

Figure 9 Bottles were kept in a laminar flow hood to avoid contamination.

2.3 Analysis

During extraction and analysis, all work concerning microbial communities was conducted in a laminar flow hood to minimize the risk of contamination. All solutions was prepared using Milli-Q water (18.2 MΩ·cm). All solutions used during extraction and analysis concerning microbial communities were sterilized in an autoclave 2x20 min at 120 ° 1 bar.
To examine how the soil conditions and microbial community structure changed with the different treatments, all analyses were conducted before and after the experiment. The production of CO_2 was analyzed continuously throughout the experiment.

Chemical characterization of soil

2.3.1 Soil moisture and water-holding capacity

The soil moisture of fresh soil was determined by weighing the sample before and after heating to 105°C over night. Water-holding capacity was determined by weighing air-dried sample before and after full wetting of soil, both analyses were performed in triplicates. Soil moisture was obtained to normalize the results per gram of dry soil for the fresh soil analyzes. Water-holding capacity was obtained to calculate amount of watering needed to keep the soil moisture at 60-75% of water-holding capacity during the experiment.

2.3.2 Gas sampling procedure

During the experiment, the rate of CO_2 production in incubation bottles was analyzed once a week to follow the soil respiration. To allow for a build-up of CO_2 and collection of headspace gas, all bottles were sealed with septa and aluminum caps 2 hours before analysis. To obtain the production rate per hour, all results were corrected with the time difference between closing of the bottle and actual analysis.

2.3.3 CO_2-production measurements

The CO_2 measurements were carried out on a gas chromatograph (GC) with a flame ionization detector (FID) (SRI 8610C). 3 ml of gas sample was collected from the bottle with a syringe pre-flushed with N_2 after which the sample was loaded on a 1000 µl injection loop. CO_2 was separated from the matrix gas on a 1.5 m 10'x1/8" stainless steel column packed with a HayeSep D 100/120 mesh at 60°C. N_2 was used as carrier gas at 4 bars and 20 ml min^{-1} flow rate. Both methanizer and detection temperature were set to 330°C. CO_2-standards of 100 ppm, 1500 ppm and 10 000 ppm (Air Liquide) were used to construct a calibration curve and were injected before and after analysis of samples, in the middle of each analysis (after 30 samples) control injections were made with the 1500 ppm standard.

2.4 Procedure for extraction of soil samples with water

For extraction of soil samples for determination of soil solution pH, total dissolved organic carbon (DOC) total dissolved N, total dissolved P, dissolved siderophores and LMMOAs and dissolved Fe/Al/Si; air-dried soil was extracted with Milli-Q water in the ratio of 1:6. The

soil-water suspensions were shaken on a reciprocal shaker; rigorously for 2 hours followed by soft shaking overnight. After shaking, suspensions were centrifuged for 10 minutes at 5000 rpm and subsequently filtered with a 0.2 µm filtropur syringe filter (Sarstedt). Samples for analysis of dissolved elements were kept at 4°C until analysis, samples for analysis of siderophores and LMMOAs were kept in a freezer until freeze drying.

2.4.1 Determination of soil pH

pH was analyzed in soil extracts both before and after the experiment. The measurements were performed using a pH-meter from Schott instruments, Lab850. Prior to analysis, the pH-meter was calibrated for pH 4, 7 and 10 with buffer solutions from Scharlau, all with an uncertainty of ± 0.01.

2.4.2 Determination of total dissolved organic carbon, dissolved nitrogen and dissolved phosphorus

To determine the total content of DOC in soil solution, samples were analyzed on a Shimadzu TOC-V_{CPH}. For analysis of total content of total dissolved N and P, samples were analyzed using a Technicon AutoAnalyzer 2. Both analyses were performed at the Department of Applied Environmental Science, ITM, at Stockholm University.

2.4.3 Determination of LMMOAs and siderophores

Before analysis of LMMOAs and siderophores, all samples (10 ml) were concentrated by freeze drying and subsequently dissolved again in 1 ml of Milli-Q water.

Quantification of LMMOAs was performed by High Pressure Liquid Chromatography (HPLC) following the method of Van Hees et al. (1999). Injection volume was set to 50µl. Samples were run on a Supelcogel C-610H ion exclusion column using a mobile phase of 0.2 % (85%) H_3PO_4 at a flow rate of 500 µl min^{-1}. The column was operated at 30 °C for detection of oxalic, tartaric, malic, maleic, malonic, succinic and fumaric acid and at 70 °C for detection of citric, pyruvic and lactic acid. The acids were detected at 210 nm by a photodiode array detector. Before analysis, all samples and controls were acidified with concentrated (85%) H_3PO_4 to a final concentration of 2.5 % H_3PO_4. As a standard for detection and quantification, a stock solution containing the 10 different acids was prepared in concentrations of 5000, 3000, 1000, 500, 100, 50, 10, 5 and 1 µmol L^{-1}. The detection limit was set to 3x S/N.

For quantification of siderophores, samples were analyzed on a column switching HPLC-MS method that was a modification of the method by Duckworth et al. (2009). The HPLC system from Thermo Scientific (Ultimate 3000 RS) consisted of two pumps; one low pressure pump operated at 30 µl min^{-1} and one high pressure pump operated at 150 µl min^{-1} and an oven compartment (Dionex Ultimate 3000, Thermo Scientific, USA) set to 10°C. The HPLC system was connected to a TSQ Quantum Access Max mass spectrometer (Thermo Scientific, USA) which detected individual siderophore hydroxamate Fe-complexes by selected ion

monitoring in positive mode (SIM) ($[M+H]^+$; Ferrichrome type siderophores (m/z): ferrichrome= 741.2; ferricrocin= 771.3; ferrichrysin= 801.2 and ferrioxamine type siderophores (m/z): ferrioxamine B= 614.2; ferrioxamine D= 656.3; ferrioxamine E= 654.3; ferrioxamine G= 672.2). For analysis, the injection volume was set to 100 µl. To pre-concentrate the analytes from the matrix, samples were loaded on a Syncronis C18 5µm pre-column (50 mm x 2.1 mm, 1.7 µm, Thermo Scientific, USA). The analytical column (for separation of the pre-concentrated siderophores) used was a Hypersil GOLD aQ (100 x 2.1 mm, 3µm, Thermo Scientific, USA). A mobile phase (A) consisting of a methanol:11mM ammonium formate buffer pH 4; (99:1 v/v) was used to enrich siderophores and was pumped through the pre-column at a flow rate of 30 µl min^{-1}. After 10 minutes, the flow switched to unload analytes from the pre-column onto the analytical column by flushing the column with a gradient of mobile phase B (Mob. B) (acetonitrile:11mM ammonium formate buffer pH 4; 15:85 v/v) and mobile phase C (Mob. C) (acetonitrile:11mM ammonium formate buffer pH 4; 5:95 v/v) at a flow rate of 150 µl min^{-1}. The gradient was set as following: 0-5 min, 80% Mob. C and 20% Mob. B; 5-15 min, 80-20% Mob. C and 20-80% Mob. B; 15-30 min 20% Mob. C and 80% Mob. B; 30-40 min 20-80% Mob. C and 80-20% Mob. B; 40-50 min, 80% Mob. C and 20% Mob. B. As a standard for detection and quantification of ferrichrome type and ferrioxamine type siderophores, a stock solution containing ferricrocin, ferrichrysin, ferrichrome, ferrioxamine B, ferrioxamine D, ferrioxamine E and ferrioxamine G was prepared in concentrations of 5, 15, 25, 50, 100, 250 and 500 nmol L^{-1}. The detection limit was set to 3x S/N.

2.4.4 Determination of dissolved Fe, Al and Si

After extraction and filtering, samples were acidified to 1% with suprapur HNO_3 before storage at 4°C. For determination of soil solution Fe, Al and Si, samples were analyzed by using inductively coupled plasma-optical emission spectrometry (ICP-OES) (iCAP 6500 Duo, Thermo Scientific). Detection limits; Fe and Al (0.5 ppb), Si (1 ppb).

2.5 Procedure for extraction of soil samples with $BaCl_2$ and Tamm´s solution

To extract soil samples for analysis of exchangeable cation content and amorphous Fe and Al oxides, air-dried soil was extracted with either $BaCl_2$ or Tamm's solution in the ratio of 1:10, respectively. Tamm's-suspensions were kept in dark to prevent the dissolution of crystalline forms of Fe and Al oxides. Suspensions were put on a reciprocal shaker for 2 hours of rigorous shaking after which they were centrifuged for 10 minutes at 5000 rpm and then filtered with a 0.2 µm filtropur syringe filter (Sarstedt). All samples were kept at 4°C until analysis.

2.5.1 *Determination of dissolved cations, exchangeable and amorphous iron and aluminum*

To extract soil for exchangeable Fe and Al, a 0.1 M $BaCl_2$ was prepared by dissolving 22.62 g of $BaCl_2 \cdot 2H_2O$ (Merck) into 1 L of Milli-Q water in a volumetric flask, following the method of Gillman and Sumpter (1986). For extraction of amorphous Fe and Al, 12.61 g of oxalic acid (Fluka analytical) and 24.9 g of ammonium oxalate (VWR) were dissolved into 1 L of Milli-Q water, giving a concentration of 0.14 M oxalic acid and 0.2 M ammonium oxalate (the so called Tamm's solution) (Jeanroy, 1983). Extraction by oxalate dissolves all Al and Fe bound to imogolite and allophane including reactive forms of Al (Gustafsson, 1998).

After extraction and filtering, samples were acidified to 1% with HNO_3 suprapur before storage at 4°C. For determination of exchangeable and amorphous Fe and Al, samples were analyzed with inductively coupled plasma-optical emission spectrometry (ICP-OES). Detection limits; Fe and Al (1 ppb).

Biological characterization of soil

2.5.2 *Microbial activity*

The enzymatic activity (i.e. microbial activity) of the microorganisms in soil was analyzed with a spectrophotometric method where the enzymatic cleavage of fluorescein diacetate (FDA) (fig 10) to fluorescein turns the samples fluorescent, following a method by Green et al. (2006) optimized for the samples in question.

A potassium phosphate buffer was prepared by dissolving 2.07 g KH_2PO_4 (Kebo) and 3.04 g of K_2HPO_4 (Merck) into 100 ml of Milli-Q water in a volumetric flask after which the solution was sterilized by autoclaving. An FDA reagent with a concentration of 0.24 mmol L^{-1} was prepared by dissolving 100 mg of FDA ($C_{24}H_{16}O_7$, Sigma Aldrich) into 100 ml of reagent grade acetone.

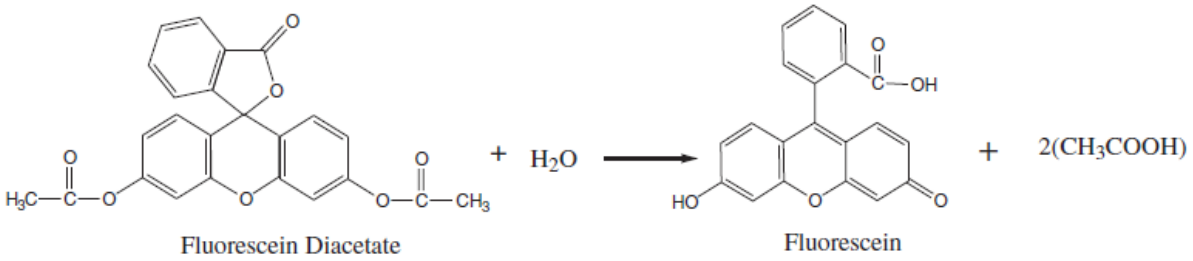

Figure 10. Fluorescein diacetate can be hydrolyzed by several enzymes which results in the production of fluorescein, measurable by spectrophotometry (Green et al., 2006).

For analysis, 49.5 ml of phosphate buffer and 500 µl of FDA was added to fresh soil in sterile 50 ml tubes, 100 mg for the O- and E horizon and 300 mg for the B- and C horizon. The mix was shaken for 2 hours in the dark after which 20 ml of the solution was transferred into new tubes. Tubes were centrifuged at 6000 rpm for 5 minutes after which the optical density of the samples was measured at 490 nm on a spectrophotometer from Hitachi (U-1100). A fluorescein standard stock solution with a concentration of 0.30 mmol L^{-1} was prepared by dissolving 100 mg fluorescein ($C_{20}H_{12}O_5$ free acid, Sigma Aldrich) into 100 ml of reagent grade acetone in a 100 ml volumetric flask. The solution was used to create a set of standards where the stock solution was diluted 15, 30, 45, 100, 150, 200, 500, 1000, 2000 and 3000 times to create a calibration curve for the FDA analysis.

2.5.3 Enumeration estimated by most probable number of cultivable bacteria

The number of viable cells present per gram of soil was quantified before and after the experiment by enumeration. Extracts were attained following the same procedure as was undertaken during extraction for inoculation. For the analysis, a ten-fold dilution series of the supernatant was achieved by diluting 1 ml of supernatant into 9 ml of 8 ‰ NaCl, creating 6 different dilutions (10^{-2}-10^{-7}). Using a multistep pipette, 25 µl of each dilution was transferred into 24 wells each of a 96-well microtitre plate, together with 200 µl of Luria Broth (Sigma Aldrich). Plates were then incubated at 24°C for 72 h. Optical density was measured at 590 nm using a spectrophotometer from Bio-Tek at 0 h and 72 h. All wells that after correction with blank had an optical density greater than 0.1 were considered positive (Balland-Bolou-Bi & Poszwa, 2012). To attain the Most Probable Number (MPN) of cultivable bacteria, the results from the inoculation of different dilutions was processed using the MPN calculator (EPA, version 2.0) with a confidence interval of 95 %. All liquids used for the analysis was first sterilized in an autoclave for 2 x 20 minutes at 120°C 1 bar.

2.5.4 Community level physiological profiling (CLPP)

To characterize the composition of the microbial communities, both in fresh soil from Norunda but also after the experiment, community level physiological profiles (CLPPs) were created using a Biolog EcoPlate™ (Biolog Inc., Hayward, CA, USA) (fig 11). These consist of 96-well microtitre plates that contain the 31 most important C substrates for soil community analysis and a water control, all in replicates of three. A redox indicator of tetrazolium dye is also included in each well, which allows the utilization of a certain C source by the microbial community to be indicated by the well turning purple (Garland, 1996). The microbial community was extracted from fresh soil following the same procedure as was undertaken during extraction for inoculation and enumeration. 1 ml of supernatant was diluted in 9 ml of 8 ‰ NaCl, from which 1 ml was again diluted into 9 ml of 8 ‰ NaCl, creating a final dilution of 1:1000 (Classen et al., 2003). Using a multistep pipette, 150 µl of the dilution was transferred into each well of an EcoPlate. To assess the purple color development due to C utilization, the optical density of plates were read at 590 nm every day during a range of 1 to 5 days on a microplate reader from Bio-Tek. To ensure an even color distribution in each well, the plate was agitated before analysis. Wells that had an optical

density greater than 0.25 after correction with water blank were considered positive (Garland, 1996). All liquids used for the analysis was first sterilized in an autoclave for 2 x 20 minutes at 120°C and 1 bar.

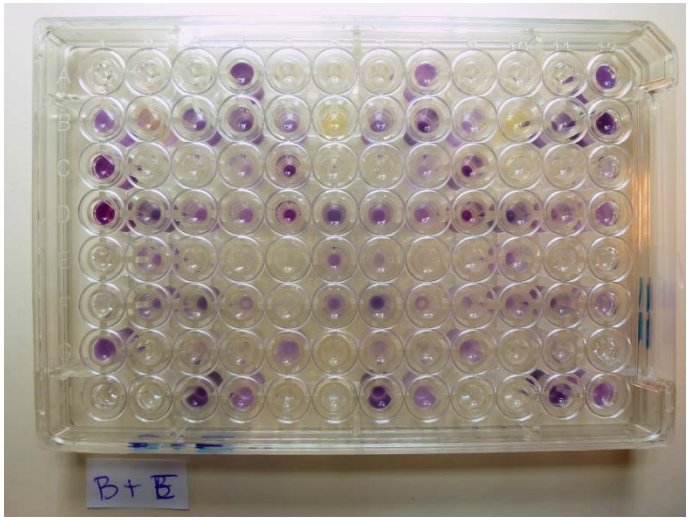

Figure 11. The purple color formation in the wells of the EcoPlate indicates utilization of a certain C source by the inoculated microbial community.

2.5.5 Statistical analysis

The values for all chemical and biological parameters are calculated as an average between three replicates with standard deviation (except for samples where concentrations were zero). Significant differences between extracts for each horizon and parameter was determined by one-way ANOVA followed by Tukey-Kramer HSD test with a 95% confidence interval (n=3). Multivariate correlation was performed on all parameters analyzed for each horizon (except for CO_2) with a 95% confidence interval (n=12). The software used for statistical analyzes was JMP® 10.0.0.

3. Results

In the following presentation of results, the samples named “initial soil” refer to the initial condition in sterilized soil, before the experiment. The samples named “abiotic control” refer to the incubated and sterilized soil to which only sterilized Milli-Q water was added. Samples named “O” “E” “B” and “C” refers to the horizon to which the extract added belongs.

3.1.1 Sterilization

Table 2 shows the percentage increase or decrease in each parameter after sterilization. In general, the O- and E-horizon appears to be more affected by the sterilization compared to the B- and C-horizon. The parameters which further experience the greatest change are DOC, dissolved N and dissolved P (for chemical characterization before and after the experiment, see appendix A1 table 1).

Table 2. The percentage change of all parameters analyzed after sterilization.

Parameter	O horizon	E horizon	B horizon	C horizon
	% Change			
pH	-8	-6	-4	-1
DOC	242	217	140	0
Dissolved P	233	250	25	-8
Dissolved N	192	300	-50	-74
LMMOAs	-3	18	39	63
Ferrichrome	-	-	-	-
Ferrioxamine	-	-	-	-
Dissolved Fe	38	23	-22	-25
Dissolved Al	96	47	-9	0
Dissolved Si	9	-21	-25	-22
Exchangeable Fe	3	43	59	55
Exchangeable Al	-15	-10	-2	8
Amorphous Fe	-5	43	-2	-7
Amorphous Al	-8	54	-2	-8

3.1.2 Soil respiration

The CO_2 efflux from soil was analyzed once a week during the three months of incubation to follow the soil respiration rate. The general trend among the extract treated soil and abiotic controls is that the highest respiration rates are found in the treatments with the O-horizon (fig 12) and the lowest in the C-horizon (see appendix A2 for figures for the E-, B- and C-horizon). During the first two weeks, there is a higher efflux in both extract treated soil and abiotic controls. After approximately 3 weeks, the respiration in the O-, E- and B-horizon has

decreased and starts leveling out whereas the respiration in the C-horizon reaches this condition already after approximately 2 weeks.

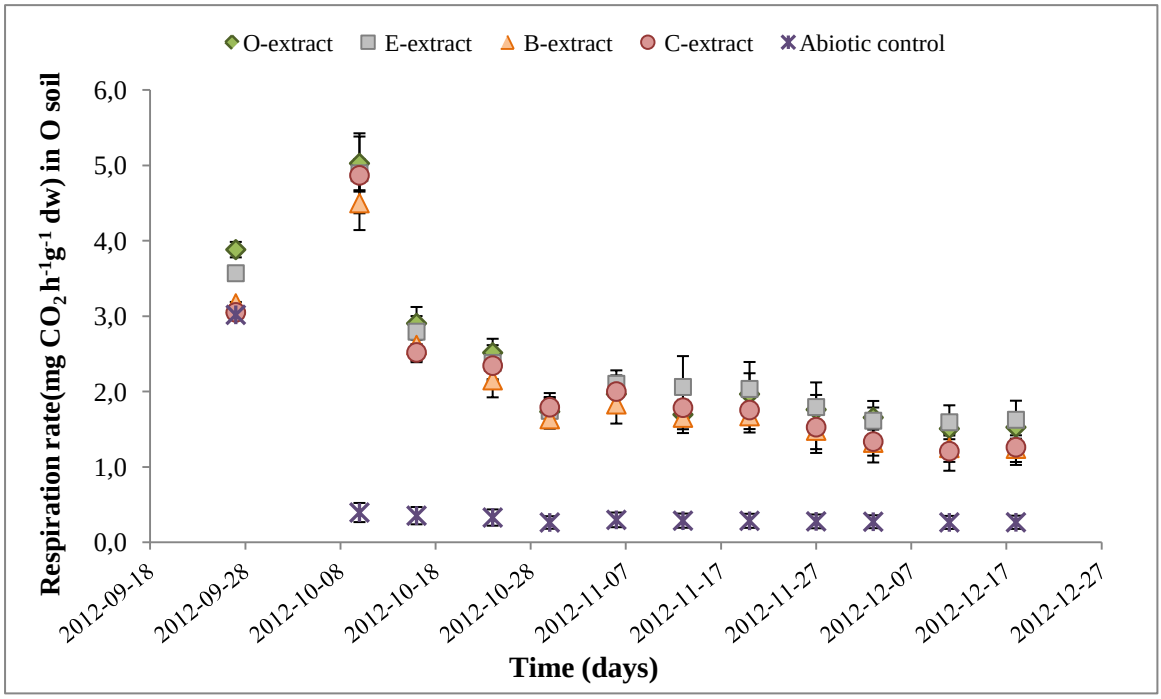

Figure 12. The respiration rate was analyzed in each incubation bottle once a week during the three month incubation. The graph represents the CO_2 production in bottles with O-horizon during the experiment where each symbol represents O-horizon treated with O-, E-, B- or C-horizon extract and an abiotic control with only sterilized Milli-Q water added. The respiration rate was measured in mg CO_2 produced per hour per gram of dry weight of soil. The respiration is calculated as the average between three bottles and the bars represents the standard deviation. (For the figures for the E-, B- and C-horizon, see appendix A2).

Following the respiration in the O-horizon soil, incubations with O-horizon extract have the highest rate of CO_2 production followed by E-, B- and C-horizon extracts at the first sampling. Also abiotic controls have similar production rates of CO_2. After two weeks, the respiration rates are at its highest concentration for all four treatments (around 5 mg $h^{-1}g^{-1}$) where O-horizon extracts average at a somewhat higher rate than the other treatments. The abiotic controls are significantly lower than samples with extracts added and are down to levels below 0.3 mg $h^{-1}g^{-1}$ where it stays throughout the experiment. Three weeks into the experiment the CO_2 production rates start to decrease and are now below initial respiration rates for all four treatments. The CO_2 production rates continue to decrease until the end of the experiment with no significant difference between the different extracts.

As for in the O-horizon soil, the O-horizon extracts have a higher respiration rate, also in the E-horizon soil at the first sampling followed by E-, B-, C-horizon extracts and the abiotic controls (appendix A2, figure 1). The highest respiration rate (1.6 mg $h^{-1}g^{-1}$) for the O-horizon extracts is measured at the first sampling occasion, whereas the treatments with E-, B-, and C-horizon extract reach their highest respiration rate after two weeks (also around 1.6 mg $h^{-1}g^{-1}$) after which it decreases. Abiotic controls show significantly lower CO_2 production rates than

the extract treated soil and decreases down to levels below 0.2 mg $h^{-1}g^{-1}$ where respiration rates remain unchanged until the end of the experiment. After 6 weeks, the E-horizon extracts averages at a slightly higher respiration rate compared to O-, B- and C-horizon extracts (only with significant difference to B-extracts and controls). Between week 6 and week 12, the E-horizon extracts continuously average at a higher respiration rate followed by O-, C- and B-horizon extracts, however without any significant differences.

In the treatments with B-horizon soil, respiration is generally much lower than in the O-horizon and E-horizon soil. At the first sampling, the C-horizon extract shows a significantly higher respiration (0.29 mg $h^{-1}g^{-1}$) than the O-, E- and B-horizon extracts ($p<0.05$) whereas the abiotic controls averages below 0.1 mg $h^{-1}g^{-1}$ already from the beginning (appendix A2, figure 2). At the second sampling, respiration in all treatments has decreased to around 0.1 mg $h^{-1}g^{-1}$. Throughout the rest of the experiment respiration for all extracts averages below 0.05 mg $h^{-1}g^{-1}$ without any significant difference between different extracts. Abiotic controls average significantly lower at 0.04 mg $h^{-1}g^{-1}$ ($p<0.05$).

In bottles with C-horizon soil, the CO_2 production rate at the first sampling point is even lower than in the B-horizon, averaging just below 0.15 mg $h^{-1}g^{-1}$ for all four treatments; abiotic controls begin at 0.1 mg $h^{-1}g^{-1}$ (appendix A2, figure 3). During week 1-2, 4 and 9 the respiration rate in the abiotic controls are significantly lower than in the extract treated soil ($p<0.05$) and in opposite there is no significant difference present between controls and treatments during week 7, 8 and week 10-12. From the second week and forward, all treatments average between 0.035-0.055 mg $h^{-1}g^{-1}$ without any significant differences among extracts.

3.1.3 Soil solution pH

The general trend for initial soil, abiotic controls and extract treated samples is an increase in pH with horizon depth (fig 13). For initial soil pH, the O-horizon averages around pH 3.43, E-horizon pH 3.66, B-horizon pH 4.69 and C-horizon pH 5.05. After the experiment, the O-horizon abiotic controls exhibit a lower pH compared to initial soil conditions ($p<0.05$). This is also true for the average pH values of the abiotic B- and E-horizon soil experiments but without statistical significance. The pH for the C-horizon abiotic control is in opposite higher after the experiment ($p=0.022$).

For the biotic O-horizon experiments, there is a significant increase in pH for all treatments compared to both the initial soil pH and pH in the abiotic controls, there is however no statistically significant difference present between the treatments with the different extracts ($p > 0.05$, $p=0.37$). The pH for the biotic treatments of E-, B- and C-horizon soil show the same trend as for the O-horizon, with a higher pH at the end of the experiment compared to the pH in abiotic controls and the pH in initial soil, again without any significant differences among different extracts.

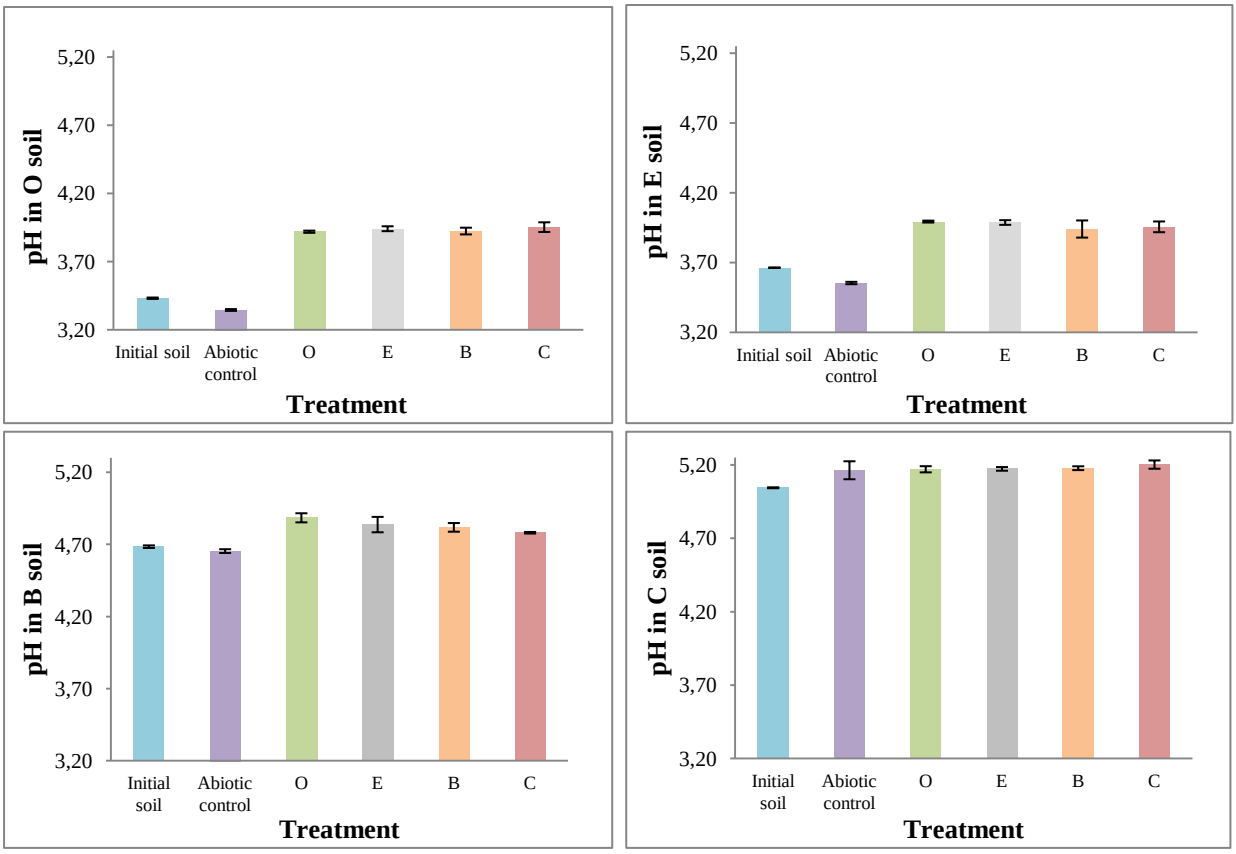

Figure 13. Before the experiment, soil solution pH was analyzed to obtain the initial value (O-, E-, B-, C-horizon before). After the experiment, pH was again analyzed on abiotic controls and on soil from the different treatments (e.g. O-horizon + O-horizon extract). The pH values showed in the figure was calculated as the average between three samples and the bars represent the standard deviation.

3.1.4 Total dissolved organic carbon, nitrogen and phosphorus

The general trend is the same for the initial soil, the abiotic controls and the extract treated samples with a decrease in the content of total DOC, dissolved N and dissolved P with soil horizon depth (table 3). In the O-horizon, extract treated samples averages at around 320 µmol DOC, 18 600 nmol N and 1850 nmol P per gram of dry weight soil. In the C-horizon, the same concentrations are 6 µmol DOC, 220 nmol N and 1 nmol P per gram of dry weight soil.

In the O-horizon soil samples, the abiotic control contains concentrations of DOC, dissolved N and P significantly higher than those present in samples treated with the four different biotic extracts ($p<0.05$). Among the extract treated samples, the lowest concentration of DOC, N and P is found with the O-horizon extract. For dissolved N this level is significantly lower than for the E-, B- and C-horizon extracts. For DOC the highest concentration is found with the B-horizon extract, a concentration which is significantly higher than with the O- and E-horizon extracts. The B-horizon extract also contain the highest level of dissolved P which is significantly higher than the P level with the O-horizon extract. For N the highest

concentration is found with the C-horizon extract. The concentrations of N are further significantly different between all extracts except the B- and C-horizon extract.

In the E-horizon soil, the abiotic control again holds the greatest concentration of DOC. The highest level of N and P is found in the initial soil. Comparing the abiotic control with the extract treated samples, these is a significant difference for DOC and N for all samples; however there is no significant difference in this aspect for P. Common for all treated samples are that the highest concentration of each analyte is found with the C-horizon extract. There is however no significant difference between the different biotic treatments.

Table 3. The concentration of total dissolved C, N and P (<0.2 µm) was analyzed in initial soil, abiotic controls and soils treated with O-, E-, B- and C-horizon extract. The concentration was calculated as µmoles per gram of soil, dry weight.

		DOC_{TOT}	N_{TOT}	P_{TOT}
Horizon	Extract	µmol g^{-1} soil dw < 0.2 µm	nmol g^{-1} soil dw < 0.2 µm	
Initial condition	O	449 ± 13	14919 ± 909	2273 ± 109
Abiotic control	Milli-Q	615 ± 20	27569 ± 136	2100 ± 19
O	O	320 ± 2	16482 ± 803	1708 ± 36
	E	329 ± 15	18149 ± 725	1872 ± 76
	B	386 ± 14	19871 ± 253	2015 ± 106
	C	343 ± 34	20001 ± 321	1818 ± 89
Initial condition	E	160 ± 6	11690 ± 991	409 ± 16
Abiotic control	Milli-Q	264 ± 74	4089 ±	196 ± 4
E	O	100 ± 13	4564 ± 344	186 ± 13
	E	92 ± 12	4794 ± 436	187 ± 11
	B	128 ± 7	4549 ± 677	215 ± 56
	C	138 ± 12	5105 ± 306	226 ± 6
Initial condition	B	11.9 ± 0.3	321 ± 135	5.8 ± 0.0
Abiotic control	Milli-Q	13.7 ± 1.1	718 ± 66	2.4 ± 0.2
B	O	9.2 ± 0.7	608 ± 4	3.4 ± 0.4
	E	9.4 ± 0.9	521 ± 30	2.8 ± 0.4
	B	9.0 ± 0.3	505 ± 79	2.1 ± 0.0
	C	12.0 ± 3.4	507 ± 89	2.1 ± 0.2
Initial condition	C	4.5 ± 0.0	168 ± 22	2.2 ± 0.3
Abiotic control	Milli-Q	6.7 ± 1.0	286 ± 109	1.6 ± 0.6
C	O	6.1 ± 0.2	227 ± 15	1.2 ± 0.1
	E	6.0 ± 0.8	219 ± 7	1.1 ± 0.1
	B	5.3 ± 0.6	204 ± 12	1.0 ± 0.1
	C	5.1 ± 0.3	212 ± 4	1.1 ± 0.1

Concentrations represent the average and standard deviation from three replicates.

In the B-horizon soil, the highest DOC and N concentrations are found in the abiotic control. For P, the greatest concentration is found in the initial soil. For all three analytes, concentrations among extract treated samples are very similar and levels are in general much

lower compared to those of the E- and O-horizon soil. For DOC, the only significant difference is between the abiotic control and the soil treated with O-, E- and B-horizon extract. For N, the B- and C-horizon extracts holds concentrations significantly lower than that of the abiotic control and for P; there are no significant differences at all.

In the C-horizon soil, P levels are even lower than in the B-horizon soil and there is no significant difference between treated samples nor between treated samples and abiotic controls ($p>0.05$). For N, there is no significant difference present at all ($p>0.05$). For DOC, the initial soil, biotic and abiotic samples all show similar concentrations.

3.1.5 LMMOAs in soil solution

Considering the trend for the initial samples and the abiotic controls, the concentration of dissolved total LMMOAs decrease with soil horizon depth (table 4). This is in general also true for the extract treated samples from horizon O to B; however, in horizon C the concentrations are slightly higher again. In the O-horizon, extract treated samples averages at 260 nmol/g of dry weight soil, in the E-horizon at 40 nmol/g of dry weight soil, in the B-horizon at 20 nmol/g of dry weight soil and in the C-horizon at 100 nmol/g of dry weight soil. For E-, B- and C-horizon soil, the concentration in the initial samples are lower than in the abiotic control whereas the initial conditions in O-horizon soil show a higher concentration. The C-horizon is the only horizon in which treated samples (O- and B-horizon extract) contain a higher amount of LMMOAs compared to the initial conditions.

For both the O- and C-horizon soil, the highest concentration among treated samples is found with B-extract followed by O-, C- and E-horizon extract. For both the E- and B-horizon soil, the highest concentration is found with O- and E-horizon extract. Analyzing the differences in the biotic samples, there are no significant differences among the four different extracts in either horizon among extract treated soils The abiotic control however contain a significantly higher concentration of LMMOAs ($p<0.05$) compared to all the biotic samples in all four horizons.

Considering individual LMMOAs, oxalic acid is present in considerably higher concentrations; however it was only detected in 4 samples (out of 24). Following citric acid, the LMMOAs which are present in the highest concentrations are oxalic, succinic and lactic acid. Even though the total concentration of LMMOAs in general is highest in the O-horizon for all four extracts, the only acids which are present are maleic, succinic and lactic acid. The horizon which in general contain the widest range of different LMMOAs in extract treated samples is the C-horizon, where oxalic, malic, maleic, succinic, fumaric and pyruvic was detected with all extracts (except for succinic acid with the E-extract).

Moreover, it was found that only a minor part of the DOC is made up by LMMOAs which ranges between 0.08 and 0.11% for the different treatments for the O-horizon soil, for the E-horizon between 0.02 and 0.06%, for the B-horizon between 0.17 and 0.26% and for the C-horizon between 0.84 and 3.91%. There are no significant differences between different

extracts in neither horizon. For the O-, E- and B-horizon, the abiotic controls however contain significantly higher percentages of LMMOAs compared to the biotic treatments.

3.1.6 Siderophores in soil solution

For dissolved siderophores, the total amount of determined ferrichrome type siderophores are in general more frequently detected in the samples than the total amount of determined ferrioxamine type siderophores (see table 4; for individual siderophores see appendix A3, table 2) and in general, the concentration in extract treated samples decreases with horizon depth.

In the O-horizon, ferrioxamines are present neither in the initial soil nor in the abiotic control, they are however present in all extract treated soil samples. Compared to ferrichromes the concentration of ferrioxamines is lower in all extract treated samples. In the E-horizon soil, ferrioxamines are completely absent whereas ferrichromes are present in all samples including the abiotic control. In the B-horizon, both ferrichromes and ferrioxamines are present in the abiotic control and with the B- and C-horizon extract treated soil. In these samples the concentration of ferrioxamines exceeds that of ferrichromes. In comparison, all extract treated samples for the C-horizon soil contain both ferrichromes and ferrioxamines where the concentrations of ferrioxamines were highest.

Focusing on ferrichromes and considering all samples, the highest concentration is found in the O-horizon soil. Regarding the different biotic extracts in the O-horizon, the highest concentration of ferrichrome is found with the C-horizon extract, closely followed by the B- and O-horizon extract whereas the O-horizon treated with E-horizon extract contains the lowest concentration. In line with the O-horizon, the lowest concentration in the E-horizon is found with the E-horizon extract, the highest is found with the B-horizon extract closely followed by the C- and O-horizon extracts. In both O- and E- horizon soil, the abiotic control is lower than both initial and treated samples (except for the E-extract in E-soil). In the B-horizon, ferrichrome is as mentioned above only found with the abiotic control and B- and C-horizon extract. In the C-horizon, it is only the extract treated samples that contain ferrichome and here, concentrations decrease in the order of O-; B-; E-; C-horizon. However, there is no significant difference present between the biotic treatments in any horizon. The levels in the O-, B- and C-horizon extract treated soil of the O-horizon are however significantly higher than the abiotic control.

Regarding ferrioxamines, they are only present in the O-, B- and C-horizon; however, not in any of the initial soil samples. In the O-horizon, ferrioxamines are only present in extract treated samples and among these, it is the soil treated with the O-horizon extract that contains the highest concentration, followed by the C-, E- and B-horizon extracts in decreasing order. As mentioned above, ferrichromes are only present in the abiotic control and soil treated with B- and C-horizon extract among which the control shows the lowest concentration. As for ferrichromes, the highest amount of ferrioxamines was found with the O-horizon extract in

the C-horizon soil. The lowest concentrations of ferrioxamines are found with the C-horizon extract which also shows a slightly lower concentration than the abiotic control. Regarding significant differences, there is none except in the B-horizon where the soil with B-horizon extract show a significantly higher concentration compared to the abiotic control.

Table 4. The concentration of dissolved LMMOAs and siderophores in initial soil, abiotic controls and extract treated soil samples in mol per g of dry soil. Note that all acids *except* citric acid (µmol/g) are given in nmol/g, siderophores are given in pmol/g. All concentrations (including total LMMOAs) are calculated as and average (*n*=3) with standard deviation.

Horizon	Extract	Total (nmol/g)	Oxalic (nmol/g)	Malic (nmol/g)	Maleic (nmol/g)	Succinic (nmol/g)	Fumaric (nmol/g)	Tartaric (nmol/g)
Initial condition O		418812 ± 169126	395 ± 87	944 ± 96	134 ± 13	1574 ± 351	7.3 ± 0.6	96 ± 11
Abiotic control	Milli-q	248371 ± 29205	918 ± 61	-	4.5 ± 7.2	2533 ± 1467	7.2 ± 0.0	-
O	O	262 ± 101	-	-	-	126[b,c] ± 28	-	-
	E	135 ± 1	-	-	0.7 ± 0.6	134[a,c] ± 1	-	-
	B	425 ± 123	-	-	3.0 ± 0.3	238 ± 108	1.2[a] ±	-
	C	221 ± 73	-	-	5.6 ± 0.8	89[c] ±	-	-
Initial condition E		1554 ± 684	448 ± 104	417 ± 74	28 ± 42	913[a,b] ± 991	5.6 ± 3.9	34 ± 12
Abiotic control	Milli-q	2797 ± 238	505 ± 428	136 ± 76	2.4[a,c] ± 1.3	1672[b,c] ± 120	7.8 ± 2.4	-
E	O	57 ± 6	17.3 ± 1.9	36 ± 7	2.2 ± 0.5	-	1.2[a,b] ± 0.5	-
	E	46 ± 9	14.7 ± 1.2	28 ± 9	2.3 ± 0.4	-	1.6 ± 0.0	-
	B	28 ± 31	12.4[a] ±	-	2.9 ± 0.6	-	1.5 ± 0.1	-
	C	25 ± 35	-	-	3.4 ± 0.7	-	1.6 ± 0.4	-
Initial condition B		78 ± 56	28 ± 1	-	<Q.L.	-	1.0 ± 0.0	10.9 ± 3.6
Abiotic control	Milli-q	123 ± 11	50 ± 5	<Q.L.	<Q.L.	54 ± 7	1.7 ± 0.0	-
B	O	24 ± 1	11.8 ± 0.1	<Q.L.	<Q.L.	-	<Q.L.[b,c]	-
	E	24 ± 4	11.9 ± 0.4	<Q.L.	<Q.L.	-	<Q.L.	-
	B	16.5 ± 5.8	11.5 ± 0.0	<Q.L.[b]	<Q.L.	-	<Q.L.	-
	C	20 ± 6	11.4 ± 0.2	<Q.L.[b,c]	<Q.L.	-	<Q.L.	-
Initial contidion C		78 ± 3	15.2 ± 1.3	-	<Q.L.[b,c]	-	<Q.L.	7.9[b,c] ± 1.3
Abiotic control	Milli-q	107 ± 82	18.5 ± 5.3	40 ± 5	<Q.L.	142[a] -	<Q.L.	-
C	O	82 ± 44	12.1 ± 0.7	36 ± 12	<Q.L.	94[a] ±	<Q.L.	-
	E	53 ± 8	12.4 ± 0.3	40 ± 5	<Q.L.[a,b]	-	<Q.L.	-
	B	212 ± 149	12.7 ± 0.8	150 ± 195	<Q.L.	74[b,c] ± 26	<Q.L.	-
	C	75 ± 43	12.5 ± 0.3	37 ± 4	<Q.L.	73[a] ±	<Q.L.	-

[a, b, c] = replicate 1, 2, 3; denotes which replicate average and standard deviation is calculated from when the specific acid only were present in two out of three bottles; no standard deviation denotes sample where the specific acid only was present in one bottle out of three. <Q.L = below quantification limit, - = not detected.

Horizon	Extract	Citric (µmol/g)	Pyruvic (nmol/g)	Lactic (nmol/g)	% of DOC	Ferrichrome (pmol/g)	Ferrioxamine (pmol/g)
Initial condition O		415 ± 168	<Q.L.	858 ± 418	92.7	16.8 ± 4.7	±
Abiotic control	Milli-q	245 ± 28	<Q.L.	628 ± 52	40.5	11.0 ± 0.9	±
O	O	-	-	178 ± 27	0.08	93 ± 10.1	78 ± 73
	E	-	-	-	0.04	77 ± 2.5	10.8 ± 7.4
	B	-	-	184 ± 17	0.11	95 ± 53.7	5.1 ± 0.2
	C	-	-	164 ±	0.07	99 ± 33.7	14.2 ± 12.4
Initial condition E		-	<Q.L.	-	0.97	13.2 ± 3.4	±
Abiotic control	Milli-q	-	<Q.L.	162 ± 8	0.91	9.8 ± 3.9	±
E	O	-	-	-	0.06	13.1 ±	±
	E	-	-	-	0.05	4.6 ±	±
	B	-	-	57 ±	0.02	18.8 ± 6.7	±
	C	-	-	59 ±	0.02	15.5 ± 11.6	±
Initial condition B		-	<Q.L.	106 ±	0.66	±	±
Abiotic control	Milli-q	-	<Q.L.	-	0.9	0.20 ± 0.09	1.9 ± 0.5
B	O	-	-	-	0.26	±	±
	E	-	-	-	0.26	±	±
	B	-	-	-	0.18	0.74 ± 0.65	28 ± 17
	C	-	-	-	0.17	0.17 ± 0.16	5.4 ± 1.8
Initial contidion C		-	-	-	0.35	±	±
Abiotic control	Milli-q	43 ±	<Q.L.	-	1.7	±	1.1 ± 0.7
C	O	-	<Q.L.	-	1.4	1.41 ± 1.94	245 ± 233
	E	<Q.L.	<Q.L.	-	0.84	0.10 ± 0.06	6.7 ± 4.3
	B	-	<Q.L.	-	3.9	0.12 ±	2.8 ± 0.2
	C	-	<Q.L.	-	1.5	0.06 ± 0.03	1.0 ± 0.6

3.1.7 Dissolved iron, aluminum and silicon in soil solution

The concentrations of dissolved Fe, Al and Si all decreases with horizon depth for initial soil, abiotic controls and extract treated samples. The concentration of dissolved Fe in the O-horizon is approximately the same for all samples, averaging between 0.3 and 0.4 µmol/g. The highest concentration is found with the B-horizon extract which is significantly greater than those of the initial sample and the abiotic control ($p<0.05$), there is however no significant difference between extract treated samples in the O-horizon soil. The average concentration of dissolved Al in the O-horizon abiotic control (5.1 µmol/g) is more than double that of the initial soil (2.0 µmol/g) and double that of the extract treated samples (fig 14). For the extract treated samples, the O-horizon extract contains the lowest concentration of dissolved Al followed by increasing concentrations with E-, C- and B-horizon extract. There is however no significant difference between the treated samples ($p > 0.05$). For Si, both the initial and abiotic control conditions are significantly lower than for the treatments ($p<0.05$). Considering the averages, the highest concentrations are found with the C-horizon extract followed by E-, B- and C-horizon extracts.

In the E-horizon, the concentration of Fe is highest in the abiotic control sample and the initial concentrations of dissolved Fe are very similar to all biotic treatments (fig 14). The abiotic control is however only significantly different from the O- and E-horizon extract. Regarding Al, the highest concentration is found in the abiotic control which is significantly higher than both in the initial soil and extract treated samples. Considering the average values among extract treated samples, concentration is highest with the B-horizon extract followed by the C-, E- and O-horizon extracts. Moving on to Si, the concentration in the initial soil is less than half that of the abiotic and biotic samples. Considering the average values, the soil with O- and E-extract contains similar concentrations which are lower than the concentrations in the abiotic control and with the B- and C-horizon extract. Considering the standard deviation, there is however no significant difference between the abiotic controls and/or the extract treated samples.

In the B-horizon, the soil treated with the O-horizon extract contains a concentration of Fe which is significantly higher compared to those found in the soil treated with the B- and C-horizon extract ($p<0.5$). The soil inoculated with E-extract further contains a concentration of Fe which is significantly higher than the concentration found with the B-horizon extract ($p<0.5$). For Al, the abiotic control contains a concentration which is higher than the concentration in all the extract treated samples, which in turn are very similar. Except for a high concentration in the B-horizon abiotic control, the concentration of dissolved Si dominates for all treatments. The concentration of Si is further significantly higher in both the abiotic control and the extract treated samples compared to the concentration in the initial soil.

In the C-horizon, the concentrations of Si are in general much higher than those of Fe and Al. The highest concentration of Fe is found in the abiotic control which is significantly higher than those in the extract treated soil. The lowest concentration is found in soil inoculated with the B-horizon extract. As for Fe, the highest concentration of Al is found in the abiotic control

which however only is significantly higher than samples inoculated with B- and C-horizon extract. For Si there is no significant difference between samples ($p>0.05$).

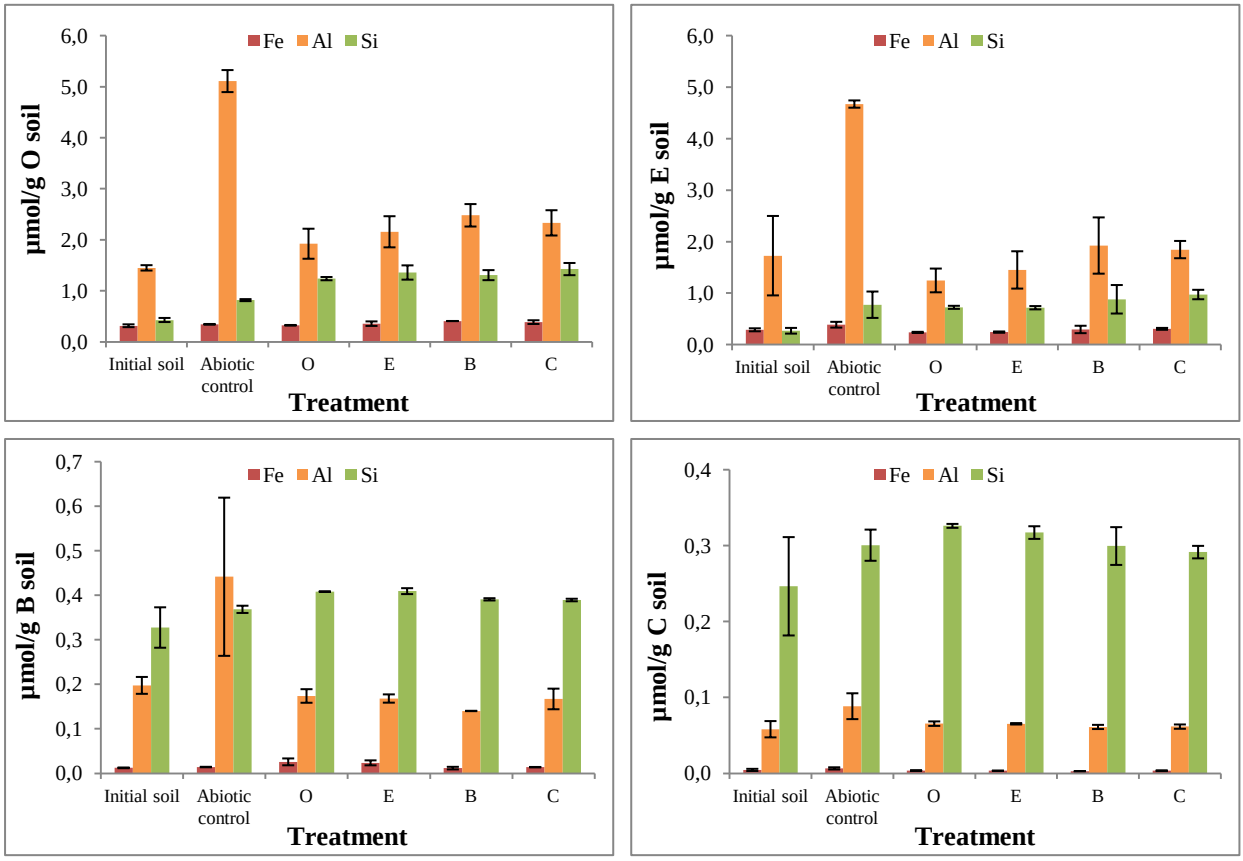

Figure 14. Concentrations of dissolved Fe, Al and Si was analyzed in initial soil samples, abiotic controls and soil treated with O-, E-, B- and C-horizon extract. Concentrations were calculated as µmoles per gram of soil dry weight and represents the average (n = 3) and standard deviation.

3.1.8 Comparison of the dissolved, exchangeable and amorphous form of Fe and Al in soil solution

The general trend for initial soil, abiotic controls and extract treated samples regarding exchangeable Fe is a decrease with horizon depth. For exchangeable Al, the highest concentrations are found in the E-horizon followed by the O-, B- and C-horizon. For amorphous Fe and Al, the highest concentrations in initial soil, abiotic controls and extract treated samples are found in the B-horizon followed by the O-, E- and C-horizon.

In the O-horizon, the amorphous form of Fe (Tamm's solution extract) dominates in all samples with a significantly lower concentration in the initial sample compared to both abiotic control and extract-treated samples ($p<0.05$) (fig 15). Regarding the exchangeable form of Fe ($BaCl_2$ solution extract) the initial soil contains the highest concentration followed by the abiotic control. Comparing the extract treated samples; the soil inoculated with O-horizon extract contains the highest level of Fe, however without any significant difference among the four different treatments. For all samples, the dissolved form of Fe is more than one order of magnitude lower than the amorphous form and more than five times lower than

the concentration of exchangeable Fe. In contrast, the largest pool of Al is present as exchangeable ions at the surface of soil particles, but in opposite to Fe, the initial soil contains a lower concentration than all other samples (however only significant for the abiotic control and O-horizon extract). The concentration of exchangeable Al with the O-horizon extract is further significantly greater than those with the E-, B- and C-horizon extract. For the amorphous form of Al, the pattern is the same as for exchangeable Al with the lowest concentration present in the initial soil. Both the abiotic control and the extract treated samples average at a slightly higher concentration compared to the concentration in the initial sample, but without any significant difference. As for Fe, the dissolved form of Al is represented by concentrations lower than both the exchangeable and the amorphous forms, in initial, abiotic and biotic samples.

In the E-horizon soil, the pattern for amorphous Fe is the same as in the O-horizon with the dominant part of Fe present in this form; however, without any significant difference between the initial, abiotic and biotic samples (appendix A4, figure 4). For exchangeable Fe, the highest concentration is present in the initial soil followed by the abiotic control. All extract treated samples average at approximately the same level, somewhat lower than the abiotic control, but due to the high standard deviation of the control it is without any statistical significance. As for the O-horizon, the dissolved form of Fe represents the smallest fraction. For Al the exchangeable form dominates with the lowest concentration found in the initial soil. The concentrations of exchangeable Al are further very similar between the abiotic control and the extract treated samples. For Al the concentration of the amorphous form is the same between all samples and always approximately three times lower than that of the exchangeable form.

In the B-horizon, it is the amorphous form of Fe that dominates, with concentrations more than 60 times higher than the exchangeable form for all samples (appendix A4, figure 5). The initial sample appears to contain the lowest concentration of amorphous Fe, there is however no significant difference between any of the samples due to the high standard deviation. For exchangeable Fe, the abiotic control holds a significantly higher concentration than all other samples. Of the treated samples, the highest concentration of exchangeable Fe is found with the O-horizon extract which is significantly higher than the concentrations with both the B- and C-horizon extract. The lowest concentration of exchangeable Fe is found in soil inoculated with the B-horizon extract, where the concentration of dissolved Fe also is slightly lower than for the other samples. As for Fe, the highest concentration for Al is found in the amorphous form, without any significant difference between samples. For the exchangeable form of Al, the abiotic control contains the highest concentration whereas the initial sample holds the lowest. There are no significant differences between the levels of exchangeable Al among extract treated samples. On the other hand, the concentration of dissolved Al is more than fifty times smaller than the concentration of amorphous Al, and 20 times lower than that of exchangeable Al.

As for the O-, E- and B-horizon, the amorphous form of Fe dominates in the C-horizon, with the lowest average concentration found in the initial soil but without any significant

difference among any of the samples (appendix A4, figure 6). Regarding the exchangeable part of Fe, treatments with O- and E-horizon extract show significantly higher concentrations than the soil inoculated with B- and C-horizon extracts. Overall, the extract inoculated soil contains concentrations lower than those found in both the initial and the abiotic sample. Following the pattern of the previous horizons, it is again the amorphous form of Al that is the dominating fraction, without any significant difference between any of the samples. Also the exchangeable form shows similar concentrations between all samples.

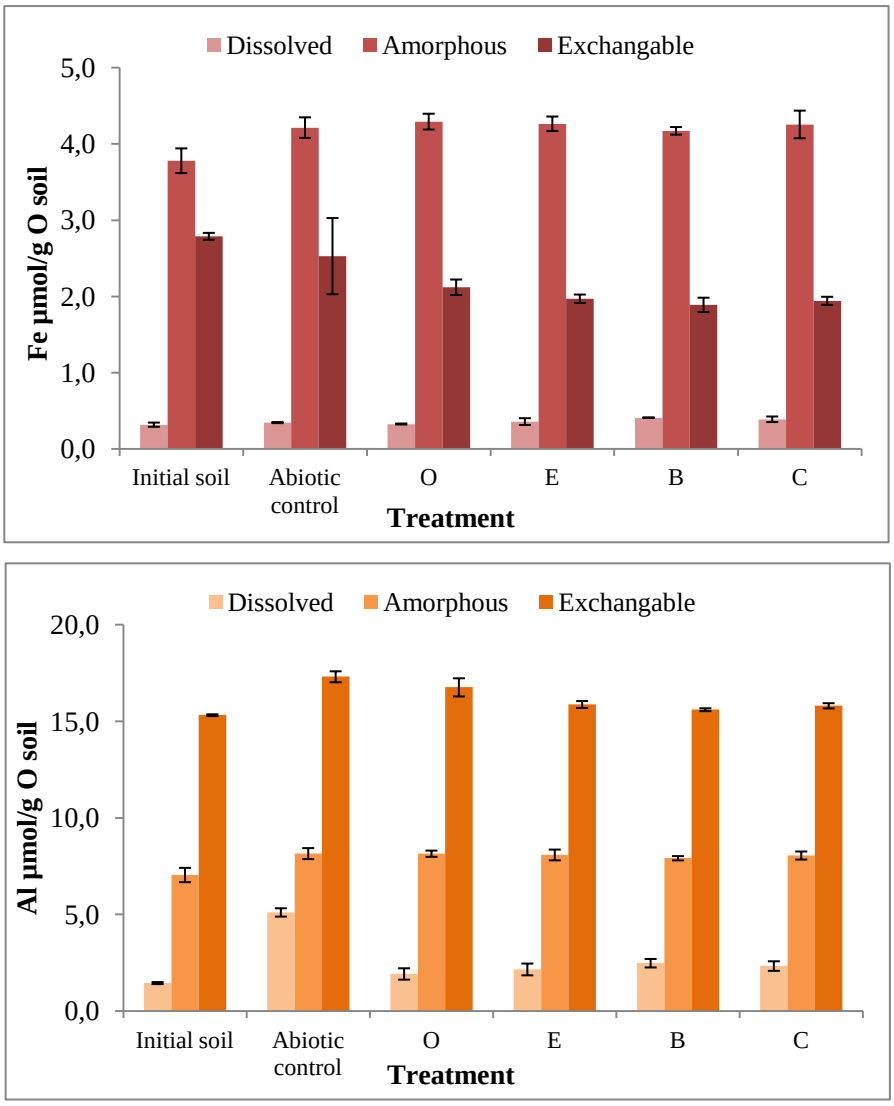

Figure 15. Dissolved, exchangeable and amorphous Fe and Al in the O-horizon soil. The elements were analyzed in initial soil samples, abiotic controls and soil treated with O-, E-, B- and C-horizon extract. Concentrations were calculated as µmoles per gram of soil dry weight. (See appendix of the E-, B- and C-horizon graph).

3.1.9 Microbial activity and number of cultivable bacteria

The microbial activity and number of cultivable bacteria was determined in fresh soil collected in Norunda in August 2012 and subsequently after the experiment in fresh O-, E-, B- and C-horizon soil treated with O-, E-, B- and C-horizon extract (table 5). The general pattern for the microbial activity in both initial soil and extract treated soil is a decrease with soil horizon depth. After the experiment, the O-horizon communities exhibit activities around 920 µg fluorescein g^{-1} dw $2h^{-1}$ whereas the activity in the C-horizon averages around 21 µg fluorescein g^{-1} dw $2h^{-1}$.

In the O-horizon, the O-horizon extract showed the highest microbial activity whereas the lowest was found for the E-horizon extract (table 6), the differences are however not statistically significant. In the E-horizon, the O-extract had a significantly ($p<0.5$) higher activity at 541 µg fluorescein g^{-1} dw $2h^{-1}$ than the other three extracts, with the lowest activity found for the C-extract (264 µg fluorescein g^{-1} dw $2h^{-1}$). In the B-horizon, it was the E-horizon extract that showed the greatest microbial activity at 47 µg fluorescein g^{-1} dw $2h^{-1}$, which also coincided with the highest number of cultivable bacteria, whereas the B-horizon extract had the lowest activity. There was however no significant difference in microbial activity between treatments in the B-horizon soil. In the samples with soil from the C-horizon, the C-horizon extract exhibited a significantly higher microbial activity (28 µg fluorescein g^{-1} dw $2h^{-1}$) than that of the B-horizon extract (15 µg fluorescein g^{-1} dw $2h^{-1}$). It was also the B-horizon extract which had the lowest activity in the C-horizon.

Initial conditions in fresh soil from Norunda were as follows; the O-horizon contained approximately 1.4×10^5 cultivable bacteria, the E-horizon 1.3×10^5, the B-horizon 1.9×10^4 and the C-horizon 1.0×10^4. The number of cultivable bacteria in the extract treated soils does not show the same declining pattern with horizon depth as the initial soil conditions and the microbial activity do. An overview of the results show that for extract treated soil, the O- and E-horizon extract contain the highest number of bacteria when incubated in the B-horizon while the B- and C-horizon extract peaks in the O-horizon.

At the end of the experiment, the largest number of cultivable bacteria in the O-horizon was found for the B-horizon extract. The number of cultivable bacteria in the B-horizon extract had increased more than 100 times compared to the initially added amount of bacteria but still coincided with the second lowest microbial activity. The lowest number of cultivable bacteria was found for the E-horizon extract which also coincided with the lowest activity. In spite the differences among the different extracts at the end of the experiment, there were no significant in the O-horizon.

In the E-horizon soil, it was the B-horizon extract which again contained the highest number of cultivable bacteria. Also here, the E-horizon extract contained the lowest number, this time over one order of magnitude lower than the number of cultivable bacteria in the B-horizon extract. There were, however, no significant differences between the four extracts.

In B-horizon, the E-horizon extract in opposite contained the largest number of cultivable bacteria and had increased more than 24 times compared to the initially added amount. The C-horizon extract, in turn contained the lowest number; however, this experiment resulted in the largest increase (almost 30 times) compared to the number of cultivable bacteria which was initially added.

Table 5. Microbial activity and number of cultivable bacteria was analyzed on fresh soil from Norunda (representing the initial condition) and at the end of the experiment on soil treated with O-, E-, B- and C-horizon extract. Microbial activity was measured as µg of fluorescein produced per gram of soil dry weight per 2 hours. Number of cultivable bacteria was measured as number bacteria per gram of soil dry weight.

Horizon	Extract	Microbial activity (µg Flourescein g^{-1} dw $2h^{-1}$)			Bacteria (MPN) (number g^{-1} soil dw)			% Change from initial addition*
Initial condition	O	730.7[a]	±	37.8	2.4×10^{4}[b]			
	E	455.8[a]	±	25.5	2.4×10^{4}[b]			
	B	23.6[a]	±	14.8	9.3×10^{3}[b]			
	C	17.3[a]	±	28.7	5.0×10^{3}[b]			
O	O	959.6	±	130.8	2.5×10^{5}	±	1.9×10^{5}	+ 942
	E	846.4	±	116.7	2.0×10^{5}	±	5.5×10^{4}	+ 733
	B	915.1	±	582.8	1.1×10^{6}	±	1.5×10^{6}	+ 11728
	C	958.5	±	448.6	4.9×10^{5}	±	7.5×10^{5}	+ 9700
E	O	541.0	±	122.0	2.2×10^{4}	±	6.5×10^{3}	- 8
	E	273.3	±	49.4	1.7×10^{4}	±	5.5×10^{3}	- 29
	B	345.0	±	41.0	5.4×10^{5}	±	7.7×10^{5}	+ 5706
	C	264.1	±	37.4	4.9×10^{4}	±	4.9×10^{4}	+ 880
B	O	30.3	±	2.4	2.7×10^{5}	±	2.9×10^{4}	+ 1025
	E	47.1	±	8.2	5.9×10^{5}	±	4.2×10^{5}	+ 2358
	B	23.2	±	17.7	1.7×10^{5}	±	7.7×10^{4}	+ 1728
	C	31.3	±	16.3	1.5×10^{5}	±	4.7×10^{4}	+ 2900
C	O	21.2	±	4.4	2.7×10^{4}	±	3.0×10^{4}	+ 1
	E	22.7	±	3.8	1.3×10^{5}	±	8.6×10^{4}	+ 442
	B	15.0	±	4.3	4.2×10^{4}	±	1.3×10^{4}	+ 352
	C	28.0	±	1.8	5.8×10^{4}	±	3.2×10^{4}	+ 1060

[a] Microbial activity in fresh soil collected in Norunda in August 2012.

[b] Approximate number of cultivable bacteria added to each incubation bottle at the beginning of the experiment.

Microbial activity and number of bacteria is the average and standard deviation for three replicates.

*Percentage increase or decrease in number of cultivable bacteria compared to the added amount ([b]).

This effect was also shown in the C-horizon, where the number of cultivable bacteria increased 10 times in the C-horizon extract. By numbers, it was the E-horizon extract that dominated in the C-horizon while the O-horizon extract was represented by both the smallest increase (+1 %) and the lowest number of cultivable bacteria. Given the large standard deviation for the incubations in the B- and C-horizons, there were no significant differences present among the different extracts.

3.1.10 Characterization of microbial community by CLPP

To obtain a fast appreciation of changes in the microbial community composition and/or function, the total number of C substrates used for cell growth was calculated. This was done both for communities extracted from fresh O-, E-, B- and C-horizon, sampled in Norunda in August 2012 (see appendix A5, table 3) and for each community extracted from the 16 different treatments at the end of the three month incubation (table 6). A comparison was then made between the four different extracts inoculated into each horizon, depending on the number of C sources each extract used. A significant difference would indicate a difference in microbial community composition and/or function. As a complement, a table showing the specific C substrates used by each extract was added, which allows for a more detailed differentiation among treatments (appendix A5, table 3). The table was categorized into polymers, carbohydrates, carboxylic acids and amino acids/amines.

Table 6. The number of carbon sources that each extract utilizes at the end of the experiment.

Horizon	Extract	Number of carbon sources	Horizon	Extract	Number of carbon sources
O	O	16 ± 2^{A}	E	O	13 ± 1^{B}
	E	19 ± 2^{A}		E	17 ± 2^{A}
	B	11 ± 1^{B}		B	14 ± $2^{A, B}$
	C	12 ± 0^{B}		C	11 ± 2^{B}
B	O	17 ± 1^{B}	C	O	17 ± $2^{A, B}$
	E	17 ± 1^{B}		E	18 ± 1^{A}
	B	16 ± 1^{B}		B	14 ± 1^{B}
	C	20 ± 1^{A}		C	17 ± $2^{A, B}$

$^{A, B}$ = treatments connected with the same letter denotes treatments without significant differences (<0.05) regarding the number of carbon sources used.

Evaluating the differences in microbial community composition and/or function in the O-, E-, B- and C-horizon of the podzol profile in Norunda by comparing the total number of C sources used; the O- and E-horizon extracts and the B- and C-horizon extracts show no significant differences among each other, neither does the O- and C-horizon extract which originates from soil surroundings with very contrasting properties. Looking at the specific

substrates used by each extract, the B-horizon extract sticks out by using fewer carbohydrates and far less carboxylic acids than the other extracts.

After the experiment, the O- and E-horizon extract and the B- and C-horizon extract inoculated into the O-horizon soil show no significant differences in the pairs. Comparing the specific substrates, it is the E-extract that sticks out by mainly using more carbohydrates than the other three. In the E-horizon, the O-, B- and C-horizon extract show no significant differences among each other; this is also the case between the E- and B-horizon extract. The O-horizon extract however uses fewer carbohydrates than the other three, while the C-horizon extract uses far less amino acids/amines than the other three. In the B-horizon, the C-horizon extract uses significantly more C sources compared to the other extracts. Comparing specific substrates, the B-horizon extract however uses far less carboxylic acids while the O-extract uses less amino acids/amines than the other three. In the C-horizon, the only extracts which are significantly different to each other is the E- and B-horizon extract. Here the O- and B-horizon extracts however stick out for the same reasons as in soil from horizon B.

3.1.11 Pairwise correlation between parameters

Pairwise correlation was performed on all parameters (except soil respiration rate), mainly to reveal any positive or negative relationships to microbial activity, but also to reveal relationships between different parameters (appendix A6, table 4). Because the parameters showed none or little differences for extract treated samples, these could be considered belonging to the same group and so the pairwise correlations were performed for each horizon on all 12 samples.

The largest number of correlations between different parameters was found in the O-horizon followed by the B-, C- and E-horizon. Significant correlations between the number of cultivable bacteria for each horizon and the different parameters were only found in the O- and B-horizon whereas significant correlations to microbial activity was found in the O-, B- and C-horizon. Consequently, there were no correlations to either microbial activity or number of cultivable bacteria found in the E-horizon.

4. Discussion

4.1.1 Sterilization

The electron beam radiation method used for sterilization of soil in this study resulted in a change of the chemical properties of the soil. Sterilization of polymers by electron beam has previously been reported to lead to molecular bond reactions such as chain breaks (Silindir and Özer, 2009), which in this case probably lead to a higher release of nutrients from the soil. Most probably due to the higher amount of organic material, the chemical properties of the O- and E-horizon were more affected than the properties of the B- and C-horizon. Since a large part of soil organic material consist of C, N and P, these were the parameters which were most affected. The high nutrient release reduces the possibility of applying the results from this study directly on the chemical soil properties at the Norunda field site. Because the nutrient status of soils can vary greatly, not only with geographical location but also due to seasonal changes (Sillanpää, 1992), it is still applicable in the more general aspect. The effect from the sterilization was taken into consideration when evaluating the results from this study.

4.1.2 Soil respiration and the microbial influence on dissolved organic carbon, nitrogen and phosphorus

Soil respiration, which can be used as a general index for microbial activity (Nordgren et al., 1983) was measured once a week during the experiment. The decline in availability of nutrients such as DOC, dissolved N and dissolved P with horizon depth (i.e. O, E, B, C), which are normal for podzolic soil, (Gupta and Rorison, 1975; Fierer et al., 2003) is most probably the explanation for the parallel decline in soil respiration rate with increasing horizon depth. At the start of the incubation, the extract treated soils for all four horizons (including abiotic controls of the O- and E-horizon) experience a higher soil respiration, which could be explained by decomposition of dead microorganisms (Griffiths et al., 2000) and other easily degradable compounds available after sterilization.

Considering the different extracts; the lack of significant difference in respiration rate suggests that all extracts are equally capable of using the different substrates available in each horizon at the prevailing conditions and during the time frame of three months. When investigating the effect of goethite and gibbsite on the composition of microbial communities originating from the O-horizon of a forest soil, Heckman et al. (2012) also found that even though there was a change in microbial community composition, there were no changes in respiration rate between different treatments. They also concluded that their study including previous ones (e.g. Griffiths et al. 2000) found that C decomposition and mineralization rates remained unchanged even though there were significant changes in environmental parameters, suggesting an adaption of the microbial communities.

The lack in significant differences between respiration rates in this experiment also agrees with the fact that the levels of DOC show no significant differences depending on the extract incubated in soil from the E-, B- and C-horizon. In the O-horizon, the soil treated with B-

horizon extract however contain levels of DOC significantly higher than the soil treated with O- and E-horizon extract, which coincides with a slightly greater concentration of total dissolved LMMOAs with the B-horizon extract. The higher levels of DOC in initial soil compared to the lower levels after the experiment in the O-, E- and B-horizon soil suggests that the main process going on here is C consumption, much likely due to the high release of easily available C (and other nutrients) after the sterilization.

The lower soil respiration in the abiotic controls of the O-, E- and B-horizon can be coupled to the higher concentration of DOC left in these bottles at the end of the experiment. The higher concentration of DOC (in all four abiotic controls) can in turn be attributed to the absence of microorganisms which otherwise would be consuming the easily available DOC and incorporating it into their biomass (Heckman et al., 2012). It should however be noted that the DOC concentrations in abiotic controls still is greater than those in initial soil for all four horizons which probably is due to an abiotic degradation of organic material, possibly also contributing to the higher DOC levels. This high abiotic degradation could further be a result of the sterilization that made the soil more easily degradable.

Regarding the concentration of total dissolved N, the lower concentration in the initial sample from the O-horizon compared to both the abiotic control and the extract treated samples indicates a release of N during the experiment. The high concentration in the abiotic control suggests that even though the levels in extract treated samples are higher than the level in the initial sample, microbial consumption is still significant or the levels in extract treated samples would be as high as in the abiotic control. This is further strengthened by the negative correlation between dissolved N and the number of cultivable bacteria (r^2= -0.87 p=0.0002), which suggests an increased consumption as the number of bacteria increases. The significantly lower concentration of dissolved N with the O-horizon extract indicates a higher consumption related to the higher microbial activity. In the E-horizon, the significantly lower concentration in the abiotic control indicates that even though there is a consumption of N (compared to the initial concentration), there could still be a release of the nutrient due to microbial actions. As for the O-horizon, there is an overall release of N in the abiotic control and in all four extract treated samples in the B-horizon. Since the abiotic control overall contains a higher level of N compared to treated samples, it is probably an abiotic release of N that dominates while the dominating process with biotic extracts is consumption or treated samples would be as high as the abiotic control. The lack of significant differences among extract treated samples and the abiotic control in the C-horizon suggests that the abiotic release could be dominating.

In opposite to DOC and total dissolved N, the concentration of total dissolved P is lower in the abiotic control compared to the concentration in the initial samples. In 1960, Lindsay and Moreno (1960) concluded that the P availability in acid soil is controlled by direct precipitation with Fe and Al. This decrease of P in the abiotic controls in this present study could therefore, based on the findings of Lindsay and Moreno (1960), be explained by a greater concentration of amorphous Fe and Al to which P could adsorb, which is the case in the O-, E- and B-horizon. The lower concentration of dissolved P in the extract treated

samples compared to initial conditions (and compared to the abiotic control in the O- and C-horizon) is most probably related to a microbial consumption of the element. The consumption of P in the O-horizon is further strengthened by its negative correlation to the number of cultivable bacteria (r^2= -0.74 p=0.006) suggesting an increased consumption as the community develops. Something which further agrees with the findings of Lindsay and Moreno (1960) is the positive correlation between dissolved Fe and dissolved P (r^2=0.72 p=0.0081) in the O-horizon. The positive correlation between dissolved Al and dissolved P (r^2=0.83 p=0.0009) in the E-horizon indicates that the concentration of P here could be governed by the dissolution of Al bearing minerals which in turn seems to be controlled by the level of DOC (r^2= 0.68 p=0.0157).

Even though there is a general lack in significant differences among the different extracts in all four horizons, there is a higher concentration of dissolved P in the O-horizon inoculated with the B-horizon extract compared to the concentration with the O-horizon extract. This could be related to the higher number of cultivable bacteria and LMMOAs with the B-horizon extract which might lead to an increased dissolution of P-bearing minerals.

4.1.3 Microbial influence on pH, LMMOAs and siderophores

Both in initial soil and extract treated soil, the general trend is a decrease in pH with soil horizon depth, a trend which corresponds to the normal conditions of podzolic soil (Degórski, 2007). The significantly lower pH in abiotic controls compared to that of extract treated samples could very well be explained a decomposition of LMMOAs in the presence of microbial extracts, whereas these compounds remain in the abiotic controls where they contribute to a lower pH. This further agrees with the fact that the abiotic controls all contain significantly greater concentrations of LMMOAs compared to extract treated soil for all four horizons. The negative correlation between pH and DOC (r^2= -0.58 p=0.0484) in the extract treated samples in the B-horizon further strengthens that the pH is affected by the presence of DOC. The DOC concentration and percentage of LMMOAs of DOC is also significantly higher in the abiotic controls compared to all the extract treated samples in the O- and E-horizons and the O-, E- and B-horizon extract treated samples of the B-horizon, something which agrees with a consumption of LMMOAs. These findings further agree with a study by Lundström et al. (1995) where they concluded that the soil solution from sterile soil columns percolated by sterile mor (O-horizon) extract (abiotic experiment) and an synthetic oxalate-citrate solution had a lower pH compared to non-sterile columns percolated with the same unsterilized mor extract (biotic experiment), explained by a microbial consumption of LMMOAs. They also found that the DOC level remained constant when solutions were percolated through sterile columns whereas a decrease in DOC occurred in non-sterile soil columns.

In the O-horizon, moderate negative correlations between both pH and microbial activity (r^2= -0.63 p=0.0295) and pH and number of cultivable bacteria (r^2= -0.60 p=0.0404) indicates that pH decreases as the community develops. This could be due to an increased production of

LMMOAs (parallel to the consumption) and increased degradation of organic material resulting in increased DOC levels. This is further strengthened by the positive correlation between the number of cultivable bacteria and DOC (r^2=0.70 p=0.0108). It should however be clarified that this negative correlation between pH and activity/number of bacteria only is true for and governs the pH in extract treated samples. In the more general aspect, comparing both abiotic and biotic systems it is the degradation of DOC and LMMOAs that to a high degree governs pH.

Comparing the concentration of LMMOAs in extract treated samples with the initial soil concentration; there is a decrease present in the O-, E- and B-horizon soil that suggests that the microbial consumption of LMMOAs is greater than the production. The high level in abiotic controls again indicates an abiotic degradation of organic material (resulting in the release of LMMOAs) parallel to the absence of microbial consumption. In the C-horizon the extract treated samples in opposite contain a greater concentration of LMMOAs compared to the initial condition, something which imply that the production of LMMOAs exceeds the consumption. It is, however, hard to discriminate between abiotically and biotically induced production since the abiotic control contains a greater concentration of LMMOAs than treated samples.

4.1.4 Microbial influence on dissolved Fe, Al and Si in relation to the presence of siderophores and LMMOAs

The trend for both the initial and extract treated soil with the greatest concentration of dissolved Fe, Al and Si in the O-horizon followed by a decrease with soil horizon depth matches the decrease of LMMOAs and the results of previous studies performed on podzolic soil (Giesler et al., 2000; van Hees et al., 2000). In the O-horizon, the O-, E- and C-horizon extracts do not seem to have a different effect on the amount of dissolved Fe, Al or Si since there are no significant differences in concentrations among the three. The higher concentration with the B-horizon extract could be related to the higher concentration of total LMMOAs and DOC. The negative correlation between dissolved Fe and the number of cultivable bacteria (r^2= -0.66 p=0.0203) in the O-horizon indicates a parallel dissolution and consumption of Fe. This would be further strengthened by a higher concentration of dissolved Fe in the control which is not the case in this study. The lower concentration of dissolved Fe in the abiotic control could however be related to the lower concentration of siderophores. The amount of Al in solution decreases very fast around pH 4 (fig 3) after which a higher pH results in precipitation of the element (e.g Al oxides). The pH of the O-horizon abiotic control averages at pH 3.4 whereas the pH in initial soil averages at pH >3.7 and extract treated samples at pH >3.9. Therefore, the significantly greater concentration of dissolved Al in the abiotic control compared to both initial soil and extract treated samples could be related back to the pH dependence of Al. The lower pH in the abiotic control can in turn be related to the higher concentration of LMMOAs due to a low degradation. Low consumption of LMMOAs could also lead to increased weathering rates in abiotic controls, releasing more nutrients into solution (Lundström, 1995). Even though there is no significant difference regarding Si

concentrations among treated samples, the concentration of both the initial soil and the abiotic control is lower. This could indicate that the biotic weathering of Si in the O-horizon is more important than the abiotic weathering for solubilization during these conditions.

As for LMMOAs and dissolved metal species, the concentration of siderophores is greatest in the O-horizon and decreases with soil horizon depth, agreeing with the distribution found in previous studies on podzolic soil (Holmström et al., 2004; Essén et al., 2006). In general, the concentrations of fungal siderophores as ferrichrome are greater than that of bacterial siderophores as ferrioxamine in all horizons, which could indicate a higher presence of fungi over bacteria, which also is normal in podzolic soil (Ekelund et al., 2001; Nikonov et al., 2001). Comparing abiotic controls to extract treated samples, the concentration of siderophores are very low or not detected at all in controls, something which is naturally explained by the absence of microorganisms. Otherwise, all siderophore concentrations lie in the range of pmol/g dry soil, which corresponds to an earlier study on podzolic soil in Sweden by Ahmed and Holmström in Ahmed (2013).

In the O-horizon, the positive correlation between dissolved Fe, Al and DOC (Fe: r^2=0.83 p=0.0009, Al: r^2=0.75 p=0.0053) indicates that DOC controls the dissolution of these elements. There is however no correlation between the dissolved species in the O-horizon with siderophores or total LMMOAs. This implicates that the correlation to DOC therefore could be the result of complexation of Fe and Al to HMM organic acids. This correlation between dissolved metal species and DOC further agrees with a previous study where they concluded that DOC determines the chemistry, the solubility and the transport of Fe and Al in the O-horizon (Hughes et al., 1990).

In the E-horizon, the very similar concentrations in dissolved Si between the abiotic control and the extract treated samples suggests that the abiotic weathering is as important as the biotic weathering of Si containing minerals in the E-horizon during these conditions. The levels of both Fe and Al peak in the abiotic control which again can be explained by the higher presence of DOC and total LMMOAs and the pH dependence of both elements. Only considering the average concentrations of Fe, Al and Si, these are highest with the B- and C-extract whereas the concentration of total LMMOAs shows the highest concentrations with O- and E-horizon extract. Both DOC and ferrichromes peak with the B- and C-horizon extract which suggests that both HMM organic acids (since the concentration of LMMOAs is low) and ferrichromes could be important for the dissolution of minerals with the B- and C-horizon extract in the E-horizon. There are no correlations between the dissolved analytes and total LMMOAs or siderophores in general in the E-horizon, but between Al and DOC and Si and DOC (AL: r^2=0.68 p=0.0157, Si: r^2=0.73 p=0.0066) which indicates that DOC is responsible for the dissolution of Al and Si.

In the B-horizon, the greater concentration of dissolved Fe with the O- and E-horizon extract coincides with a slightly higher level of total LMMOAs while siderophores in opposite only are present with the B- and C-horizon extracts, corresponding to the conditions in the E-horizon. The negative correlation between microbial activity and the concentration of dissolved Fe (r^2= -0.61 p=0.0363) may indicate a consumption of Fe in the B-horizon, it

could, however also be explained by an increased precipitation of the analyte. For Al, the concentration is again greater in the abiotic control, relatable to the higher concentration of total LMMOAs. This is further strengthened by the positive correlation between dissolved Al and the concentration of total LMMOAs in the B-horizon (r^2=0.63 p=0.0296) which again indicates that LMMOAs control dissolution of Al during these conditions.

In the C-horizon, both Al and Fe show the greatest concentrations in the abiotic control which again coincide with higher levels of total LMMOAs. The highest concentration of siderophores is found with the O-horizon extract, however, without any visible effect on dissolved analytes. The positive correlation found between Fe and DOC, Fe and ferrichromes and Fe and ferrioxamines (r^2=0.64 p=0.0247, r^2=0.66 p=0.0191, r^2=0.69 p=0.013) indicates that the small amount of dissolved Fe and Al could be dependable on the presence of HMM organic acids (because of lack in correlation to LMMOAs) and siderophores. The negative correlation between Al and pH (r^2= -0.63 p=0.0271) again confirms the dependence on pH for the speciation of Al.

4.1.5 Microbial influence on the speciation of Fe and Al

Regarding the initial and extract treated soil conditions, the amount of exchangeable (and dissolved) Fe and Al decrease with soil horizon depth, something that is normal for podzolic soil and corresponds to the decrease in microbial activity (Nissinen et al., 1998). Corresponding to the theory of podzolization, the amount of oxalate extractable amorphous Fe and Al is largest in the B-horizon where secondary minerals such as goethite and allophane are formed upon precipitation of dissolved species percolating down the soil column (Degórski, 2007).

In the O-horizon, the increase in the amorphous fraction of Fe could be explained by a precipitation of Fe from the exchangeable fraction which undergoes a parallel decrease. The decrease in exchangeable Fe can also be coupled to a microbial consumption since exchangeable ions represents an important nutrient source in soils (Ugolini et al., 2001). The negative correlation between DOC and amorphous Fe and Al (Fe: r^2= -0.77 p=0.0035, Al: r^2= -0.77 p=0.0038) further suggests that the increase of the amorphous fraction for both elements could be due to the parallel decrease in DOC which may be hindering the precipitation of Fe and Al in the initial sample. For Al, the exchangeable fraction increases with the amorphous fraction, possibly as a consequence of increasing pH.

In the E-horizon, the lack in significant differences in concentration of amorphous Fe and Al between the initial sample, the abiotic control and the extract treated samples suggest that the microbial extracts do not have an effect on this fraction during this time frame at these conditions. For exchangeable Fe, the lower concentration in extract treated samples indicates that there is a microbial consumption of the Fe. For the exchangeable fraction of Al the trend is the same as in the O-horizon which again could be explained by the increase in pH.

In the B-horizon, the lack in differences between all the samples regarding the amorphous fraction of Fe and Al again indicate a lack in effect from microbial communities. In total, the

concentration of exchangeable Fe is greatest in the abiotic control where the consumption of Fe logically should be zero. The treatments in turn have smaller concentrations of exchangeable Fe than both initial soil and the control, but a slightly higher concentration of amorphous Fe. This indicates a consumption of Fe and could also indicate that a lesser fraction of the amorphous phase is being dissolved, maybe due to the consumption of LMMOAs. This is further supported by the positive correlation between exchangeable Fe and total LMMOAs (r^2=0.65 p=0.0212), which suggests that Fe partly is controlled by the concentration of LMMOAs, that in turn is lower in extract treated samples compared to the abiotic control. The negative correlation between Fe and ferrichromes and ferrioxamines (r^2= -0.65 p=0.0233, r^2= -0.69 p=0.0137) could further explain the slightly higher concentration of exchangeable Fe with the O- and E-horizon extract where we have an absence of siderophores, which otherwise could dissolve this part of the Fe. The exchangeable fraction of Al is also negatively correlated with both siderophore groups however without any differences between the four extracts, something which is difficult to explain.

In the C-horizon there are again no significant differences among microbial, abiotic or initial soil samples regarding amorphous Fe and Al, which shows that the microbial extract do not have any significant affect in this fraction during this short incubation period, the same goes for exchangeable Al. In opposite to the O-horizon, the exchangeable fraction of Al in the C-horizon is positively correlated to the concentration of DOC (r^2=0.62 p=0.0323), maybe because of a dissolution and transformation of amorphous Al to exchangeable. The concentration of exchangeable Fe is again lower in the soil treated with B- and C-horizon extract which could indicate a slightly higher microbial consumption in these samples.

4.1.6 Changes in microbial activity, number of bacteria and metabolic profile in different horizons

The general decrease in microbial activity with soil horizon depth, both during original and experimental conditions agrees with the findings of Federle et al. (1986) who also used the FDA technique to measure the microbial activity in soil. Except for factors such as soil moisture and temperature (parameters which were kept rather constant during the present experiment), substrate availability, especially the availability of C will affect both the biomass and the activity of soil microorganisms (Schimel, 1989; Griffiths et al., 1998; Blume et al., 2002). Therefore, the parallel decrease in nutrient availability (e.g. DOC, dissolved N and P, dissolved and exchangeable Fe) could most likely be one explanation for the decrease.

Focusing on the different extracts, there are no significant differences in microbial activity in the O- and B-horizon. In the E-horizon, however, there is a significantly higher activity in the O-horizon extract which coincides with a slightly higher concentration of total LMMOAs. The higher activity is however not reflected in a higher abundance of bacteria which indicates that the activity could account for the presence of fungi. In 2003, Fierer et al. (2003) concluded that microorganisms residing in the lower part of the soil profile had a potential activity which may be as high as, or even higher than that of surface microbes, something which could result in the very similar activity of the B- and C-extract compared to the O- and

E-horizon extract in O- and E-soil. In the C-horizon, the higher activity of the C-horizon extract coincides with a slightly higher number of cultivable bacteria. This higher activity is probably related to the fact that the C-horizon extract is well equipped for the conditions of its own original soil horizon.

When correlating the microbial activity to the different chemical parameters analyzed, there is a negative correlation to dissolved Fe present in the B-horizon and a negative correlation to exchangeable Al in the C-horizon. This could indicate that the microbial activity could partly be controlling the mobilization of metals in the B- and C-horizon during these conditions. In a previous study by Gelsomino and Azzellino (2011) they found a positive statistical relationship between microbial activity and TOC (total organic carbon) and TN (total nitrogen) in soil. When comparing the results for microbial activity analyzed with different methods, they however found that results were opposite depending on the method used (i.e. FDA-hydrolysis as used in this experiment and acid and alkaline phosphatase) with FDA-method showing the highest activity in acid soil compared to alkaline soil whereas the phosphatase-method found the highest activity in alkaline soil. This demonstrates that several methods for determining microbial activity might be needed in order to fully discover the relations between microbial activity and the chemical parameters of soil.

The decrease of number of cultivable bacteria with horizon depth in initial soil agrees with a previous study by Balland-Bolou-Bi and Poszwa (2012) who also found a decrease with soil horizon depth. The trend for the number of bacteria for extract treated soil samples in the present study is however not the same as for the initial soil and the parameter of microbial activity. Comparing both the average number of bacteria and the change in number (from the extract added for each horizon), the O- and E-horizon extract both have their peak in the B-horizon. This finding is hard to relate to any of the other parameters but could maybe be related to the R-strategy and K-strategy of bacteria. This theory suggests that K-strategy bacteria originating from nutrient rich horizons (e.g. O- and E-horizon) produce low amounts of strongly chelating LMMOAs and use small amounts of C while R-strategy bacteria originating from nutrient poor conditions (e.g. B- and C-horizon) produce high amounts of weakly chelating LMMOAs and use large amounts of C (Balland-Bolou-Bi et al., 2013). Following this theory this could maybe be an explanation for the high growth of the O- and E-horizon extract in the B-horizon even though nutrient levels are lower. The B- and C-horizon extract peak in the O-horizon, perhaps because of the high nutrient availability which in turn allows for the use of large amounts of C (R-strategy). Focusing on the number cultivable of bacteria in extract treated soil there are no significant differences among treatments for any horizon because of the large standard deviation. Large standard deviation is further a problem when working with living organisms and especially with extracts from soil. This because there are many steps in the process which can affect the amount of microorganisms actually extracted. It should also be mentioned that less than 1 % of bacteria are cultivable (Øvreås and Torsvik, 1998), which leads to the fact that enumeration by MPN that was used in the present study only gives a small indication on the actual biomass.

By testing the ability of the microbial communities to utilize different C substrates, an indicator for the community structure of cultivable microorganisms was given. As for determining the number of cultivable bacteria, the CLPP method is restricted to detecting C usage by cultivable microorganisms which leads to the results not being representative of the whole soil microbial community. One should also bare in mind that the contributions of particular populations to the pattern in the Biolog plate (CLPP) may not reflect the relative proportion of the original inoculum (Preston-Mafham, 2002). Taking these facts into consideration, the use of the CLPP method still gives a crude estimation of how the microbial community is structured and if there are any changes present.

Using the very basic interpretation of counting the number of C sources used, it appears as there are not four completely different microbial communities extracted from the four soil horizons of the investigated podzolic soil from Norunda, something which however was assumed in the second hypothesis. This is also indicated by the similarities in the kind of C sources used by the different extracts. The fact that the B-horizon extract uses fewer carboxylic acids (e.g. succinic and lactic acid) compared to the other three extracts could however indicate that this community still is different from the others. The fact that the podzol profile in Norunda is quite undeveloped, having small distinct layers, could be an explanation for the close similarities between the extracts of the four horizons. In another study on community diversity and composition, Michel and Williams (2011) found that in young soils, the different soil horizons share 75% of the 16 rRNA genes and that this sharing will decrease with increased soil age and soil horizon development. They also concluded that horizon development and soil genesis are important factors in the determination of bacterial community composition and structure in soil.

Comparing the results from the fresh soil of Norunda to the result of the extracts which were inoculated into their originate horizon (e.g. O-extract inoculated into the O-horizon) one could expect these to be equal. This is however not the case as the B- and C-horizon extract uses a greater number of C substrates compared to the extracts from fresh soil, something which could be an artifact of the sterilization which lead to an increased release of nutrients.

Comparing the number of C sources used at the end of the experiment, it appears as the microbial communities inoculated into each horizon are even more alike now than before the start of the experiment. This could indicate a change in microbial community structure or an adaption of the community function to the new surroundings. This is further something which could be strengthened by, and also explain the lack of significant influence between the different extracts on several parameters (e.g. pH, LMMOAs etc.). When considering the specific substrates used, the small deviations in the metabolic profiles (kind of substrates used) between the different extracts could however be indicative of a difference in microbial community. Something which contradicts a change in community structure is the short time frame of the experiment where a previous study by Budge et al. (2011) found that the response of microbial communities in subalpine soils is slow, with only moderate shifts in community structure after 11 years. These experiments were however conducted at much lower temperatures (mean -2 – 2.3°C) than this experiment.

To be able to make more accurate interpretations, more data analysis of the CLPP results need to be performed. These include the construction of kinetic profiles for each substrate and extract which will be acquired by calculating the rate of average color development. More multivariate statistical analyses would also be needed in order to more thoroughly evaluate the substrate utilization pattern of the different extracts. Since the CLPP-method does not discriminate between changes in substrate utilization pattern due to changes in microbial community structure and/or adaption of the prevalent community and because the methods of both CLPP and enumeration only covers cultivable bacteria, more detailed analyzes are required. One method which previously was executed by Griffiths et al. (2000) is extraction and molecular analysis of RNA and DNA, which are central components when examining the diversity of microorganisms in the environment.

5. Conclusions

Going through the results for the different parameters analyzed in this experiment, the microbial communities originating from the O-, E-, B- and C-horizons of the podzolic soil in Norunda have a significant different influence, for example on the concentration of DOC, dissolved P, dissolved N and exchangeable Al when incubated into the O-horizon and on dissolved Fe when the extracts are incubated into the B-horizon. However, the extracts did not have significantly different influence on the following chemical and biological soil parameters during the prevailing conditions; soil respiration, pH, concentration of total dissolved LMMOAs, concentration of dissolved Al and Si, concentration of amorphous Fe and Al and the number of cultivable bacteria, as demonstrated by the lack of statistically significance. Adding to this, the concentration of DOC, dissolved P and dissolved N show no significant difference among the four different extracts when incubated into the E-, B- and C-horizon. Equally, the four extracts do not have a different influence on the concentration of dissolved Fe when incubated into the O-, E- and C-horizon. Therefore, the majority of these results may indicate that in general and during these conditions, the chemical properties of a podzolic soil horizon will not undergo a change as the community of the horizon changes.

These uniform responses in a number of analyzed parameters among the different soil horizon extracts could be related to the composition and structure of the original microbial communities from Norunda which by the CLPP-data are indicated not to be four communities with different microbial composition, but more alike. At the end of the experiment, the CLPP-data also indicates that the microbial communities in each horizon are alike, even more so than when extracted from fresh soil, which also could explain the uniform results. These similarities in metabolic functions could either be due to a change in community structure or due to an adaption of the microbial community to the “new” environment. If it is a case of adaption, the results from the present study indicate that it is not the microbial community that affects the chemical properties of its surrounding, but it is rather the microbial community that will adjust its functions and/or composition to the surrounding environment.

The only horizons in which significant correlations are found between the overall microbial activity and nutrients and/or metals are in the soil from the B- and C-horizon. The microbial activity in the B-horizon show a negative correlation to the concentration of dissolved Fe while the activity in the C-horizon show a negative correlation to the concentration of exchangeable Al. These results implicate that metal mobilization partly might be controlled by the activity of the microbial community in the B- and C-horizon. The lack in correlation between the microbial activity and nutrients (e.g. DOC, dissolved N and P) indicates that the microbial activity does not have a significant effect on nutrient concentrations during the prevailing conditions but that it may be mainly abiotic factors that affect these parameters at the early stage of redistribution of the microbial community.

Something which also may have had an effect on the influence that the four extracts have on chemical soil properties is the time frame. The experiment was very likely too short (three months) to allow for possible changes to a magnitude that is detectable, both in chemical

parameters but also in microbial community and/or structure. Another fact that most definitely played an important role in the outcome of the results of this experiment is the number of replicates. Since each incubation bottle more or less could be considered a separate experiment, the standard deviation between the three replicates is in many cases very large. Therefore, a larger set of replicates would have resulted in better conditions for detecting the trends in the experiment.

Although more investigations are needed, the results from this study implicates that following the mixing of the microbial communities from different soil horizons of podzolic soil, for example during disturbance through uprooting of trees, the redistribution of the microbial community will not lead to a direct effect on the chemical soil properties and cycling of nutrients in the soil.

For future studies, it would be very interesting to extract RNA and DNA and sequence, both the soil from Norunda but also in the soil from the different treatments. This way we could characterize both the natural microbial community structure and composition but also characterize the microbial community in the different treatments to see if there were any changes in community composition and structure during the three months of this experiment. This would also allow us to differentiate between the bacterial and fungal community to see if one or the other would be more prone to adapt faster to the changing environmental conditions. It would also be very interesting to perform a new experiment with a similar setup, this time for a longer duration (e.g. 1 year) and with a larger set of replicates. This would allow for a higher certainty of results, a better basis for detection of trends but also the possibility to take out samples during the experiment in order to detect changes that might occur with time.

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8. Appendix

A1. Before and after sterilization

Table 1. The average concentration between 3 samples for each parameter, before and after sterilization. Given in the following forms: DOC/dissolved N/dissolve P (mg/l), LMMOAs (nmol/g soil dw), Ferrichrome/Ferrioxamine (pmol/g soil dw), dissolved/exchangeable/amorphous Fe/Al/Si (µg/g soil dw).

Parameter	O horizon		E horizon		B horizon		C horizon	
	Before	After	Before	After	Before	After	Before	After
pH	3.72	3.43	3.89	3.66	4.9	4.69	5.1	5.05
DOC	263	899	101	320	10	24	9	9
Dissolved P	3.6	12	0.6	2.1	0.024	0.03	0.012	0.011
Dissolved N	12	35	3.5	14	1.5	0.75	1.5	0.39
LMMOAs	430100	418812	1320	1554	56	78	48	78
Ferrichrome	16.6	-	13.2	-	-	-	-	-
Ferrioxamine	-	-	-	-	-	-	-	-
Dissolved Fe	13	18	13	16	0.9	0.7	0.4	0.3
Dissolved Al	28	55	32	47	5.8	5.3	1.6	1.6
Dissolved Si	11	12	9.5	7.5	12	9	9	7
Exchangeable Fe	152	156	105	150	2.7	4.3	2	3.1
Exchangeable Al	487	414	590	530	88	86	36	39
Amorphous Fe	223	211	148	211	251	246	146	136
Amorphous Al	207	190	136	209	245	241	155	142

A2. Respiration

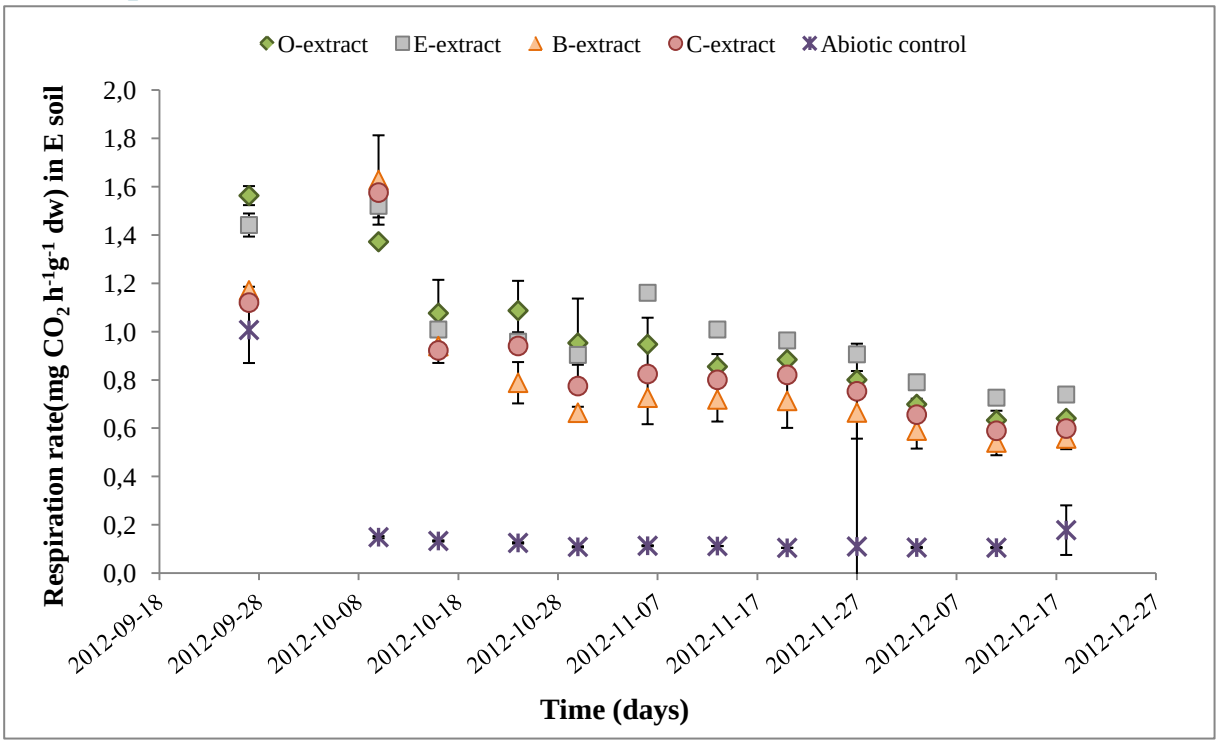

Figure 1 The respiration rate was analyzed in each incubation bottle once a week during the three month incubation. The graph represents the CO_2 production in bottles with E soil during the experiment. The rate was measured in mg carbon dioxide produced per hour per gram of soil, dry weight. The concentration is calculated as the average in between three bottles and the bars represents the standard deviation.

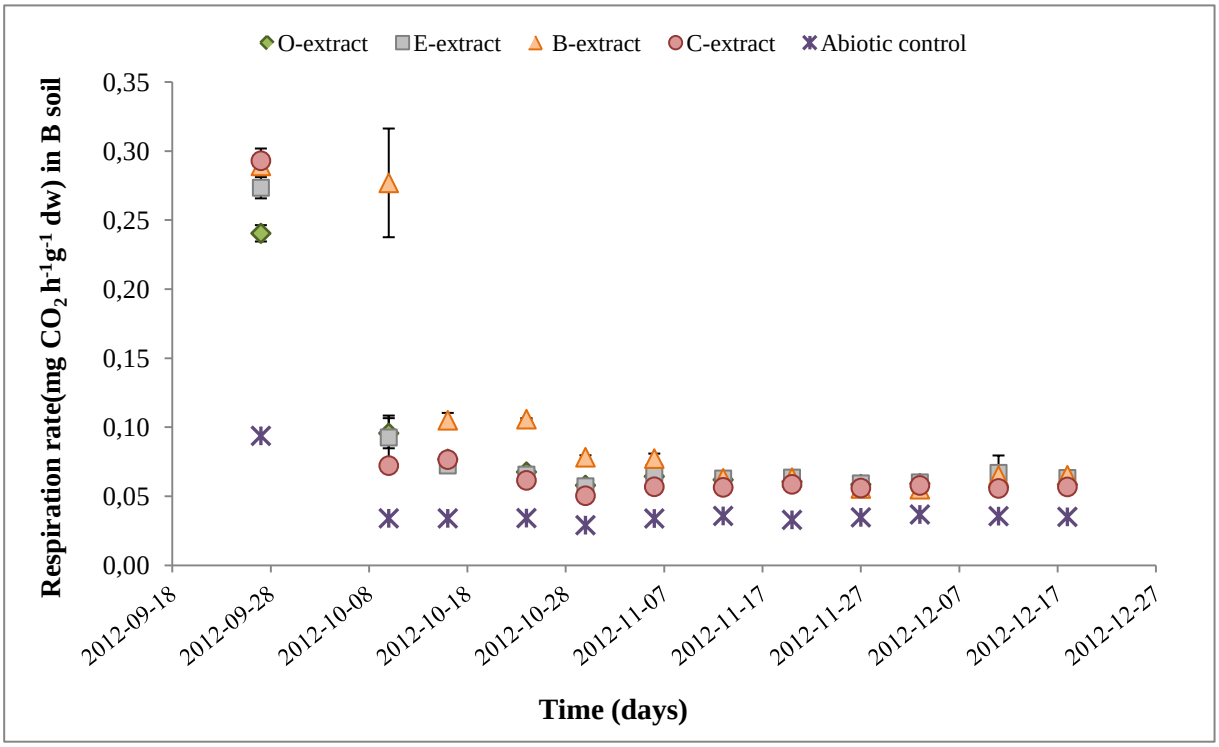

Figure 2 The CO_2 production in bottles with B soil. The rate was measured in mg carbon dioxide produced per hour per gram of soil, dry weight. The concentration is calculated as the average in between three bottles and the bars represents the standard deviation.

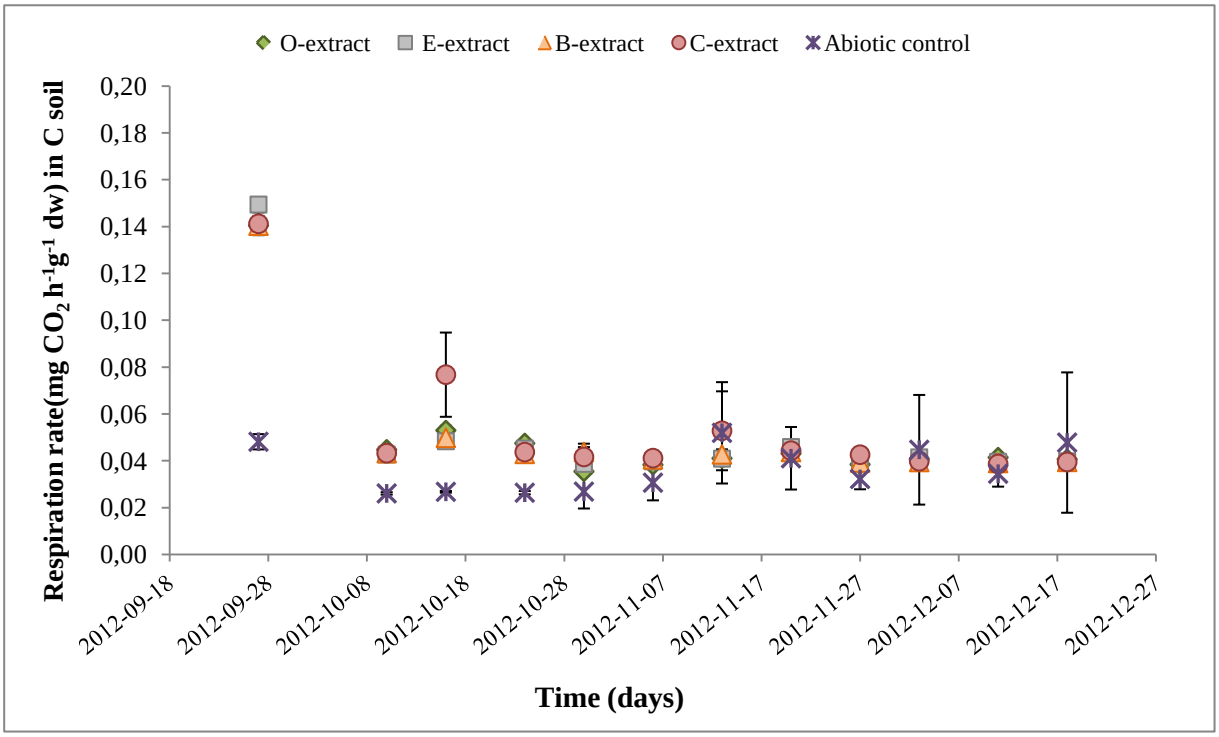

Figure 3 The CO_2 production in bottles with C soil. The rate was measured in mg carbon dioxide produced per hour per gram of soil, dry weight. The concentration is calculated as the average in between three bottles and the bars represents the standard deviation.

A3. Siderophores

Table 2. The concentration of each individual dissolved siderophore in initial soil, abiotic controls and extract treated soil sample.

Horizon	Extract	Ferricrocin (nM)	Ferrichrysin (nM)	Ferrichrome (nM)	Ferrioxamine_B (nM)	Ferrioxamine_D (nM)	Ferrioxamine_E (nM)	Ferrioxamine_G (nM)
Initial condition O		11.2 ± 10.2	-	-	-	-	-	-
Abiotic control	Milli-q	9.7 ± 0.9	1.3 ± 0.2	-	-	-	-	-
O	O	90.4 ± 10.7	1.1 ± 0.9	1.9 ± 0.5	22.6 ± 24.0	0.6 ± 0.5	1.0 ±	54.9 ± 47.8
	E	74.2 ± 1.7	1.4 ± 0.5	1.9 ± 0.1	4.2 ± 0.7	-	-	14.2 ± 5.8
	B	82.7 ± 43.6	6.8 ± 9.4	17.4 ±	39.6 ±	19.2 ± 13.4	18.5 ±	33.4 ± 16.8
	C	98.6 ± 32.5	1.7 ±	-	6.3 ± 1.2	-	<Q.L.	15.6 ±
Initial condition E		13.0 ± 3.7	-	0.7 ±	-	-	-	-
Abiotic control	Milli-q	74.4 ± 115.6	2.1 ± 0.6	2.3 ±	-	-	-	-
E	O	13.1 ±	-	-	-	-	-	-
	E	4.6 ±	-	-	-	-	-	-
	B	17.7 ± 6.5	1.1 ± 0.2	-	-	-	-	-
	C	13.9 ± 11.0	1.5 ± 0.7	-	-	-	-	-
Initial condition B		-	-	-	-	-	-	-
Abiotic control	Milli-q	0.2 ± 0.1	-	-	0.9 ±	-	-	1.4 ± 0.2
B	O	-	84.4 ±	-	-	-	-	-
	E	-	-	-	-	-	-	-
	B	0.4 ± 0.3	-	0.5 ±	16.6 ± 11.2	-	<Q.L.	11.7 ± 6.3
	C	0.2 ± 0.2	-	-	2.9 ± 1.6	-	-	2.5 ± 0.2
Initial contidion C		-	-	-	-	-	-	-
Abiotic control	Milli-q	-	-	-	0.8 ±	-	-	0.7 ± 0.1
C	O	27.0 ± 46.1	-	35.1 ±	32.9 ±	98.9 ±	45.9 ± 64.2	59.9 ± 19.4
	E	0.1 ± 0.1	-	-	2.7 ± 1.2	-	0.2 ±	3.0 ± 2.1
	B	0.1 ±	-	-	1.2 ± 0.3	-	-	1.5 ± 0.5
	C	-	-	-	0.7 ±	-	0.1 ±	<Q.L.

Concentrations are calculated as averages (n = 3) with standard deviation.

[a, b, c] = replicate 1, 2, 3; denotes which replicate average and standard deviation is calculated from when the specific acid only were present in two out of three bottles; no standard deviation denotes sample where the specific acid only was present in one bottle out of three. "-" = no detection.

A4. Dissolved, exchangeable and amorphous fraction of iron and aluminum in soil

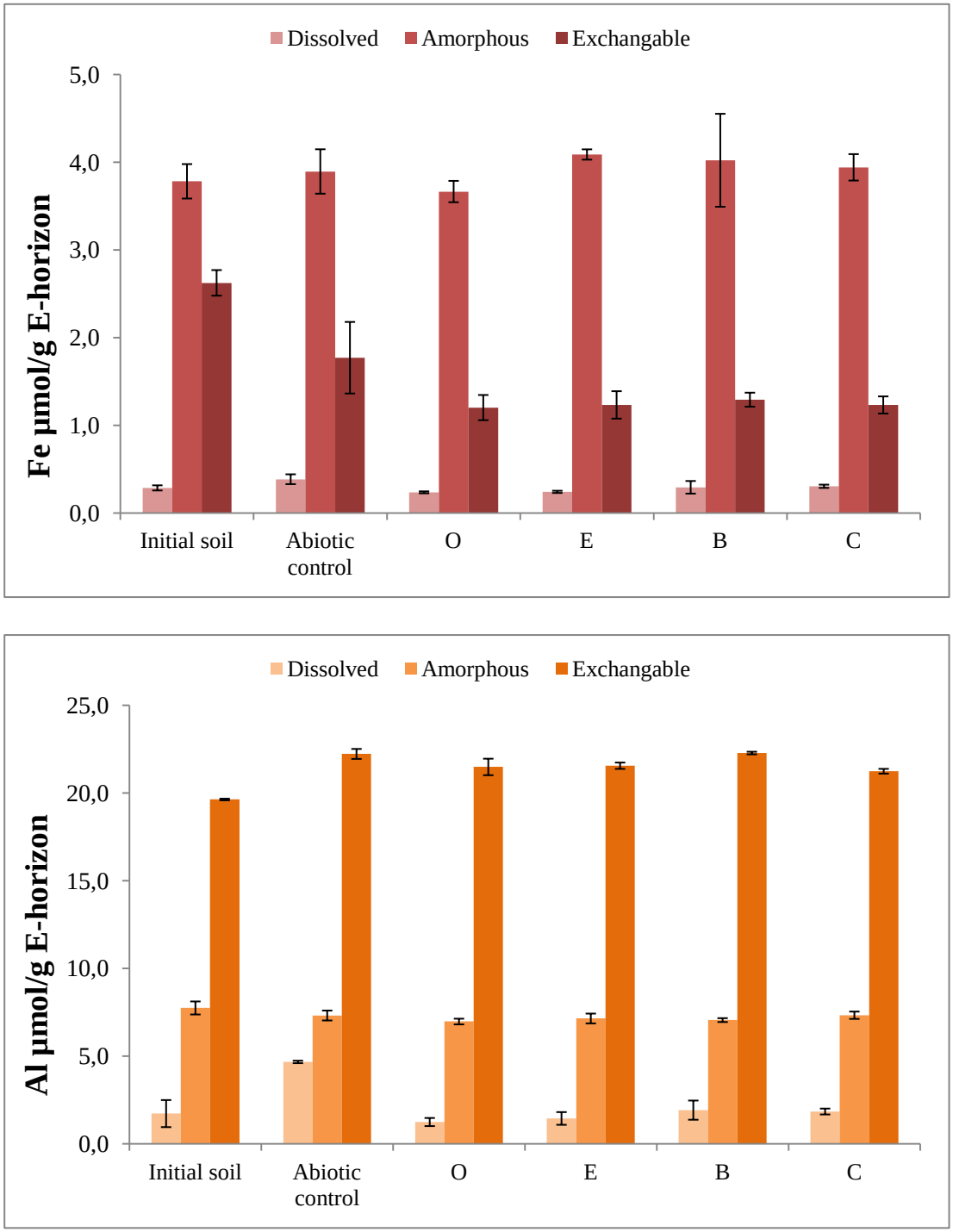

Figure 4. Dissolved, exchangeable and amorphous Fe and Al in E soil. The elements were analyzed in initial soil samples, abiotic controls and soil treated with O, E, B and C extract. Concentrations were calculated as µmoles per gram of soil dry weight.

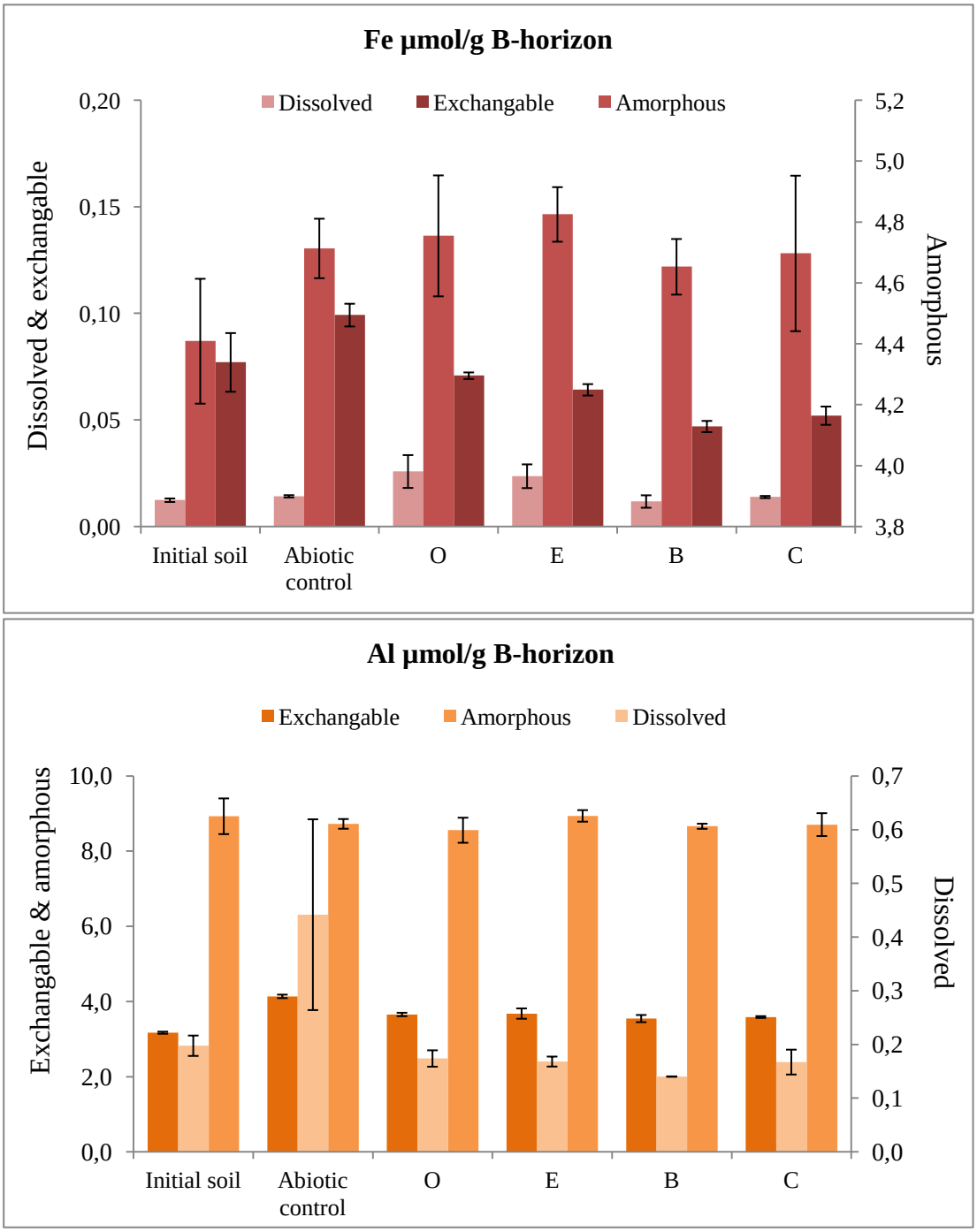

Figure 5. Dissolved, exchangeable and amorphous Fe and Al in B soil. Note that the middle bar in the Fe graph represents the amorphous fraction (y-axis to the right) and that the middle bar in the Al graph represents the dissolved fraction (y-axis to the right).

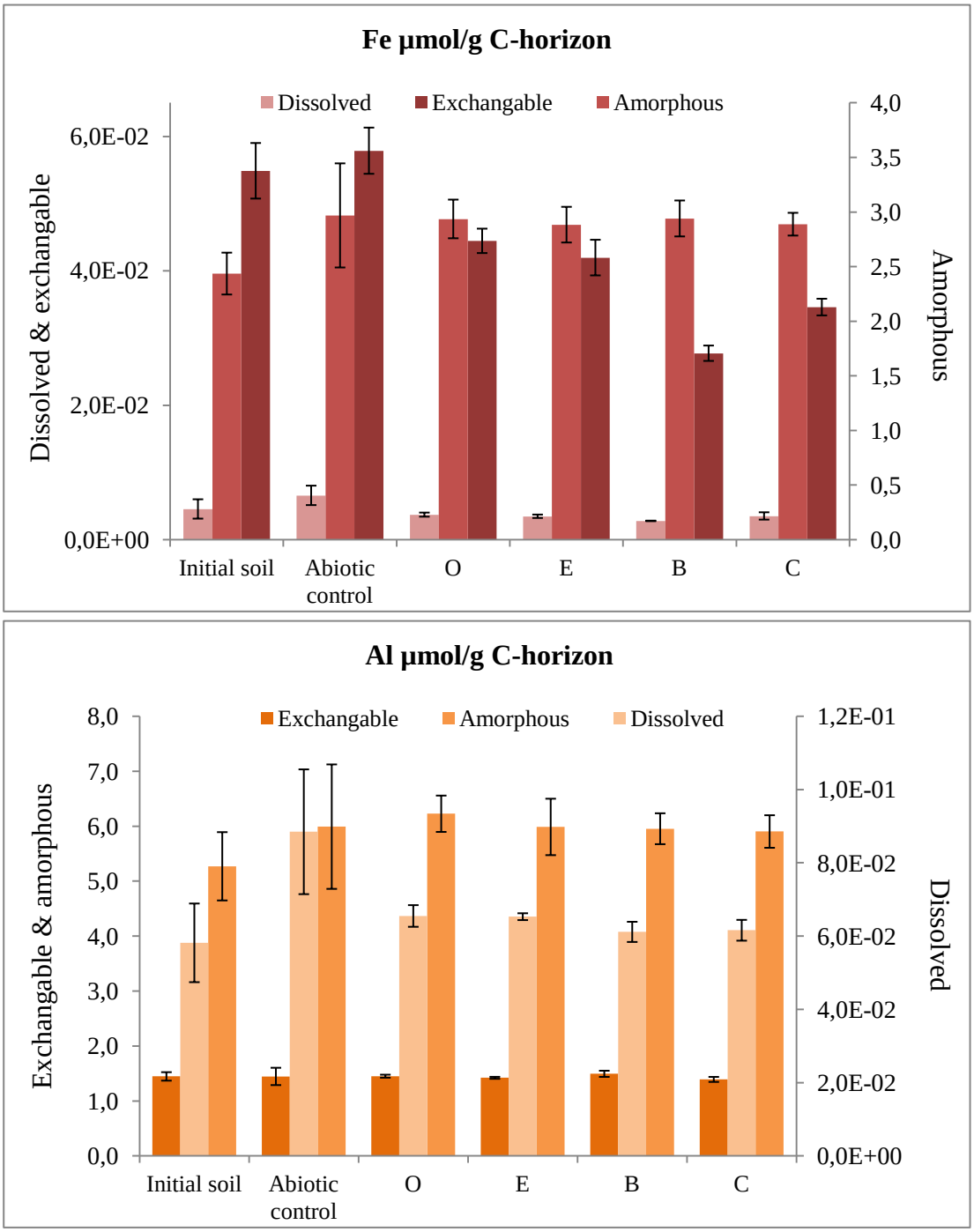

Figure 6. Dissolved, exchangeable and amorphous Fe and Al in C soil. Note that the middle bar in the Fe graph represents the amorphous fraction (y-axis to the right) and that the middle bar in the Al graph represents the dissolved fraction (y-axis to the right).

A5. Community level physiological profiling (CLPP)

Table 3 The specific carbon sources used for each extract in each horizon. "X" denotes positive response (at least 2 out of three replicates positive for the specific carbon source regarding the limit of 0.25 in optical density).

		Positive carbon sources in fresh soil*				Positive carbon sources after the experiment													
	Carbon source	Horizon				Extracts in O horizon				Extracts in E horizon				Extracts in B horizon				Extracts in C horizon			
		O	E	B	C	O	E	B	C	O	E	B	C	O	E	B	C	O	E	B	C
Polymers	Pyruvic Acid Methyl Ester	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
	Tween 40	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Tween 80	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
	Glycogen				X					X	X	X	X	X		X	X				X
Carbohydrates	β-Methyl-D-Glucoside		X				X	X			X		X								
	D-Xylose					X	X	X			X	X	X								
	i-Erythritol	X					X				X										
	D-Mannitol	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	N-Acetyl-D-Glucosamine	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
	D-Cellobiose		X			X	X		X	X	X	X	X	X	X	X	X	X	X		
	α-D-Lactose						X														
Carboxylic acids	D-Galactonic Acid ϒ-Lactone		X			X	X										X				X
	D-Galacturonic Acid	X			X	X	X	X	X	X	X	X		X	X		X	X	X		
	4-Hydroxy Benzoic Acid	X			X		X							X	X	X	X	X	X	X	X
	ϒ-Hydroxybutyric Acid													X				X	X		X
	D-Glucosaminic Acid	X	X		X	X	X	X	X	X	X	X	X	X	X		X	X	X		X
	Itaconic Acid	X	X		X									X	X		X	X	X		X
	α-Ketobutyric Acid													X							
	D-Malic acid			X										X	X	X	X	X	X	X	X
	Glucose-1-Phosphate		X																		
	D,L-α-Glycerol Phosphate	X	X																		
Amino acids & Amines	L-Arginine	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
	L-Asparagine	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
	L-Phenylalanine	X	X	X						X	X	X		X	X	X	X	X	X		
	L-Serine	X	X		X	X	X								X	X	X		X	X	X
	L-Threonine			X		X	X	X	X		X	X	X			X	X			X	X
	Glycyl-L-Glutamic Acid		X			X			X			X			X	X	X		X	X	X
	Phenylethyl-amine						X				X										
	Putrescine	X	X		X		X			X		X		X	X	X	X	X	X	X	X
Total number of carbon sources		$16^{A,B}$	18^{A}	9^{C}	$14^{B,C}$	15	20	11	12	12	17	16	10	18	17	16	20	16	18	13	18

*Positive carbon sources when analyzing the CLPP of microbial communities extracted from fresh soil sampled in Norunda in August 2012.

For total number of carbon sources in fresh soil from Norunda, horizons connected by the same letter (^{A,B,C}) are not significantly different (<0.05).

A6. Pairwise correlation

Table 4. Correlation (r^2) and significance probability (p-value) for pairwise correlation at 95 % confidence interval between the following parameters; DOC, dissolved nitrogen, dissolved phosphorus, total LMMOAs, ferrichromes, ferrioxamines, dissolved Fe, dissolved Al, dissolved Si, exchangeable Fe, exchangeable Al, amorphous Fe, amorphous Al, pH, number of cultivable bacteria and (microbial) activity. The table lists the parameters with significant correlation.

		O-horizon		E-horizon		B-horizon		C-horizon	
Parameter	by Parameter	r^2	p-value	r^2	p-value	r^2	p-value	r^2	p-value
DOC	pH					-0.58	0.0484		
Ferrichromes	pH	-0.67	0.0163						
Ferrichromes	DOC			0.58	0.0460				
Ferrioxamines	Ferrichromes					0.97	<.0001	0.98	<.0001
Nitrogen diss	DOC	0.60	0.0400						
Phosphorus diss	pH					0.67	0.0182		
Phosphorus diss	DOC	0.72	0.0088	0.59	0.0450				
Phosphorus diss	Nitrogen diss	0.65	0.0220	0.66	0.0206	0.65	0.0208		
Fe diss	DOC	0.83	0.0009					0.64	0.0247
Fe diss	Ferrichromes							0.66	0.0191
Fe diss	Ferrioxamines							0.69	0.0130
Fe diss	Nitrogen diss	0.81	0.0013						
Fe diss	Phosphorus diss	0.72	0.0081			-0.58	0.0479		
Al diss	pH							-0.63	0.0271
Al diss	DOC	0.75	0.0053	0.68	0.0157				
Al diss	Total LMMOAs					0.63	0.0296		
Al diss	Nitrogen diss	0.60	0.0413					0.85	0.0004
Al diss	Phosphorus diss			0.83	0.0009				
Al diss	Fe diss	0.81	0.0013	0.65	0.0214				
Si diss	DOC			0.73	0.0066			0.79	0.0024
Si diss	Nitrogen diss			0.67	0.0176				
Si diss	Phosphorus diss			0.89	0.0001	0.68	0.0153		
Si diss	Fe diss	0.67	0.0177			-0.73	0.0068	0.77	0.0033
Si diss	Al diss			0.79	0.0023	0.59	0.0433		
Number of bacteria	pH	-0.60	0.0404						
Number of bacteria	DOC	0.70	0.0108						
Number of bacteria	Nitrogen diss	-0.87	0.0002						
Number of bacteria	Phosphorus diss	-0.74	0.0060						
Number of bacteria	Fe diss	-0.66	0.0203						
Exchangeable Fe	Total LMMOAs					0.65	0.0212		
Exchangeable Fe	Ferrichromes					-0.65	0.0233		
Exchangeable Fe	Ferrioxamines					-0.69	0.0137		
Exchangeable Fe	Nitrogen diss					0.62	0.0326	0.75	0.0051
Exchangeable Fe	Phosphorus diss					0.85	0.0004		
Exchangeable Fe	Fe diss							0.73	0.0073
Exchangeable Fe	Al diss							0.70	0.0111
Exchangeable Fe	Si diss					0.85	0.0005	0.60	0.0396
Exchangeable Al	Ferrichromes					-0.64	0.0240		

		O-horizon		E-horizon		B-horizon		C-horizon	
Parameter	by Parameter	r^2	p-value	r^2	p-value	r^2	p-value	r^2	p-value
Exchangeable Al	Ferrioxamines	0.63	0.0289			-0.61	0.0369		
Exchangeable Al	Nitrogen diss	-0.89	0.0001						
Exchangeable Al	Phosphorus diss	-0.73	0.0069			0.64	0.0245		
Exchangeable Al	Fe diss	-0.76	0.0044						
Exchangeable Al	Number of bacteria					0.69	0.0132		
Exchangeable Al	Exchangeable Fe	0.90	<.0001						
Amorphous Fe	DOC	-0.77	0.0035						
Amorphous Fe	Ferrichromes	-0.65	0.0221						
Amorphous Fe	Fe diss	-0.69	0.0127						
Amorphous Al	DOC	-0.76	0.0038					0.62	0.0323
Amorphous Al	Fe diss	-0.76	0.0044						
Amorphous Al	Amorphous Fe	0.91	<.0001			0.63	0.0273	0.89	<.0001
Activity	pH	-0.63	0.0295						
Activity	Ferrichromes	0.60	0.0380						
Activity	Fe diss					-0.61	0.0363		
Activity	Exchangeable Al							-0.74	0.0058