BOREAL ENVIRONMENT RESEARCH 13 (suppl. B): 107–119 © 2008
ISSN 1239-6095 (print) ISSN 1797-2469 (online) Helsinki 25 November 2008

# Qualitative and quantitative changes in water-extractable organic compounds in the organic horizon of boreal coniferous forests

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*Received Apr. 2008, accepted 30 Sep. 2009 (Editor in charge of this article: Jaana Bäck)*

Hilli, S., Stark, S. & Derome, J. 2008: Qualitative and quantitative changes in water-extractable organic compounds in the organic horizon of boreal coniferous forests. *Boreal Env. Res.* 13 (suppl. B): 107–119.

In order to investigate the changes in soil organic matter along a natural decomposition gradient, we determined the concentrations and stocks of water-extractable carbon (WEC), water-extractable nitrogen (WEN), soluble phenolics and sugars in the litter (L), fermentation (F), and humus (H) layers on two site types (mesic Norway spruce and sub-xeric Scots pine forests) and in two climatic zones (north and south boreal). The WEC, WEN, phenolic and sugar concentrations decreased considerably from the L to the H layer, but the reduction was stronger in the north than in the south. Despite the highest concentrations in the L layer, the stocks of water-extractable organic matter (WEOM) were the highest in the H layer. Our investigation demonstrates for the first time that the organic stocks in the H layer outweigh the higher concentrations of water-extractable compounds in the L layer, thus making the H layer the most important stock for water-extractable compounds.

## Introduction

The water-soluble organic compounds in decomposing organic matter are considered to form the most active soil carbon pool (Kalbitz and Kaiser 2007). Therefore, despite constituting a relatively small fraction of the total amount of organic matter in the soil, these compounds have an important effect on the degradation and mineralization rates of organic matter (Miltner and Zech 1998, Gallet and Keller 1999). Due to the differences in decomposition rates among different types of substrate, the concentration of water-soluble compounds generally decreases and that of slowly decomposable compounds correspondingly increases during the course of decomposition (Berg *et al*. 1987, Berg 2000a, McTiernan *et al*. 2003, Hilli *et al*. 2008). Individual understorey plant species and tree species produce litter with different concentrations and composition of water-soluble compounds (Hobbie 1996, Gallet *et al*. 1999, Wardle *et al*. 2003, Don and Kalbitz 2005). As a result, the composition of the vegetation influences the proportions of water-soluble compounds in the decomposing litter and soil organic matter. In general, the concentration of water-soluble phenolics, for example, is higher in deciduous and evergreen dwarf shrubs in relation to that in grasses and mosses (Hobbie 1996). The concentration of water-extractable compounds is also higher in Scots pine than in Norway spruce needle litter (*see* Wardle *et al*.

2003 for comparison between the data from Berg 2000b and Berg *et al*. 2000).

The individual layers in the organic horizon of forest soils, i.e. the continuum of litter layer (L), fermentation layer (F) and humus layer (H), represent different phases in the decomposition process starting from freshly deposited litter in the L layer and ending in the formation of refractory organic matter in the H layer. Determining the changes in the chemical composition and quantity of the organic substances in a vertical direction can provide valuable information about the decomposition of the various types of substrates. Coniferous trees and dwarf shrub species produce litter that contains high concentrations of tannins and other phenolics (Gallet and Lebreton 1995, Kraus *et al*. 2004). The role of soluble phenolics in soil carbon (C) and nitrogen (N) cycling in boreal forests has received considerable attention (*see* e.g. Suominen *et al*. 2003, Smolander *et al*. 2005, Kanerva and Smolander 2008, Kanerva *et al*. 2008). However, less information is available about the stocks of soluble carbohydrates in boreal ecosystems (Miltner and Zech 1998). Phenolic compounds are probably the most stable components of dissolved organic matter (DOM), while plant-derived carbohydrates seem to be easily degradable (Kalbitz and Kaiser 2007). Studies carried out on the chemical quality of litter and organic matter have not been able to link the concentrations to the pools of these compounds on an areal basis. The quantity of water-extractable substances in soils can be expected to play a major role in the leaching of DOM from the forest floor into the underlying mineral soil, and is therefore of major importance in the C cycling of boreal ecosystems (Fröberg *et al*. 2006).

The aim of the study was to answer the following questions: (1) What are the changes in the concentrations of water-soluble C and N, phenolics and sugars down a vertical gradient from the L to the H layer in the organic horizon? (2) What is the variation in the vertical gradient of water-soluble substances along a climatic gradient between the south and north boreal zones, and between sites with different levels of site fertility (mesic sites dominated by Norway spruce and sub-xeric sites dominated by Scots pine), in Finland? (3) What is the relationship between the concentrations of water-soluble compounds and the stocks of water-soluble compounds? In order to find answers to these questions we analyzed the concentrations and stocks of water-extractable C (WEC), water-extractable N (WEN), and extractable phenolics and sugars down the vertical soil gradient in sub-xeric Scots pine and mesic Norway spruce plots situated either in the south boreal or north boreal zones in Finland. We predicted that (1) the concentrations of water-extractable compounds decrease down the vertical decomposition gradient from the L to the more decomposed material in the F and H layers; (2) because soluble sugars form the most readily decomposable substrate group in soils (*see* von Lützow *et al*. 2006), their concentration should decrease sharply from the L to the H layer, while the concentration of slowly-decomposable phenolics (Fierer *et al*. 2001, Kraus *et al*. 2003) should follow a weaker trend; and (3) because nutrient-poor conditions enhance the concentrations of phenolics in plants (e.g. Northup *et al*. 1995), the concentrations of phenolics should be significantly higher on the sub-xeric pine sites than on the mesic spruce sites.

## Material and methods

### Study area and sampling

We sampled six plots situated in the north boreal zone (pairs of mesic and sub-xeric plots in Kivalo, Sodankylä, and Pallasjärvi; hereafter referred to as “north”) and six plots situated in the south boreal zone (pairs of mesic and sub-xeric sites in Juupajoki, Tammela, and Punkaharju; hereafter referred to as “south”) for the study (Fig. 1 and Table 1). In the north boreal zone, where the growing season is shorter, the effective temperature sum smaller, and the soils less fertile (Derome *et al*. 2007a), tree growth and biomass are much lower than in the south boreal zone. In the north boreal zone sub-xeric Scots pine forests are dominant, whereas in the south boreal zone mesic Norway spruce forests are more common (Meriluoto and Soininen 1998). Eleven of the plots are a part of the EU/Forest Focus and UN-ECE/ICP Forests intensive monitoring network, with one extra plot (mesic plot in Sodankylä) not

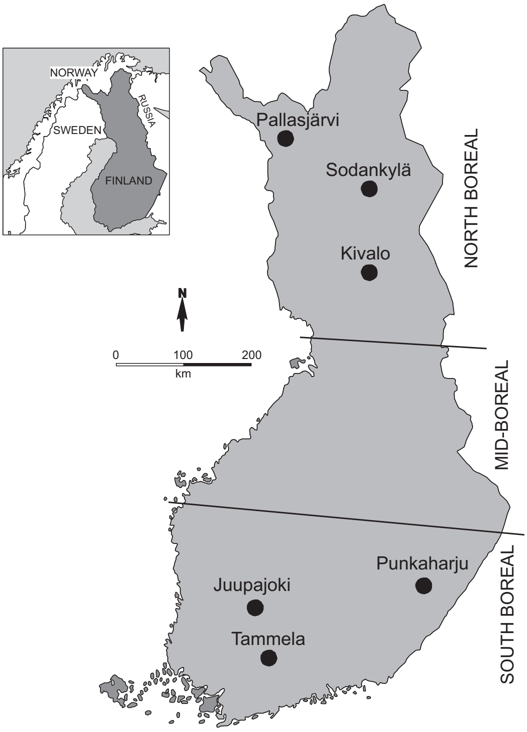

**Fig. 1**. Location of the study sites.

belonging to the network. Precipitation (during 1 June–30 Sep. 2002) was determined on nine of the plots using three precipitation collectors (diameter = 20 cm) located in open areas near to the forest plot. The collectors were emptied at 2-week intervals and the volume of rainfall determined by weighing. Precipitation was determined as the average value of the three collectors. On two of the plots, measurements made at the nearest weather station of the Finnish Meteorological Institute were used. On the Pallasjärvi sub-xeric plot, the corresponding value from the mesic plot was used. The temperature sum (threshold +5 °C) on the plots was calculated from temperature measurements made at a height of 2 m inside the stand. The temperature was recorded at 1-minute intervals by a datalogger, and averaged on a daily basis. We also used a site index ($H_{100}$), which estimates tree height at the age of 100 years, as a parameter in this study because it depicts the fertility and climatic factors such as temperature, precipitation, and their variation throughout the years. It, therefore, expresses the conditions under which litter is produced and decomposed. Vegetation cover analyses have earlier been conducted on the sites (*see* Salemaa and Hamberg 2007). The most common species in the understorey vegetation are *Vaccinium myrtillus, V. vitis-idaea*, *Empetrum nigrum*, and *Calluna vulgaris*. On the spruce plots, the cover of dwarf shrubs varies from 7% (Punkaharju) to 62% (Pallasjärvi), and the cover of mosses from 54% (Tammela) to 94% (Pallasjärvi). On the pine plots the cover of dwarf shrubs varies from 27% (Punkaharju) to 50% (Pallasjärvi), and mosses from 52% (Tammela) to 88% (Juupajoki). The average cover of herbs and grasses is generally very low (0%–0.2%), except on the mesic plots at Juupajoki (13%) and Tammela (18%). Lichens are common on the Pallasjärvi and Sodankylä sub-xeric plots.

Each study area in the intensive monitoring network consists of three plots (30 × 30 m). The plot used for assessing ground vegetation (one of the three) was used in this study (Derome *et al*. 2007a). The size of the Sodankylä mesic plot is also 30 × 30 m. A total of 28 small squares (30 cm × 30 cm) were marked out along the four edges (7 along each edge) of the 30 × 30 m square plot during mid-July to mid-August in either 2002 or 2003. The total area of the squares was 2.52 m$^2$ on each plot. Each sample square was then carefully removed so as to include all the living ground vegetation and the organic layer (L, F, H), but none of the underlying mineral soil. The squares were placed in plastic bags and stored in a freezer (–18 °C). After thawing in the laboratory, all the aboveground living (green) plant material was removed and reserved for further studies.

In the laboratory, the intact 30 cm × 30 cm samples taken from the plots were visually separated into the L, F and H layers. The L layer, which consisted of clearly distinguishable fragments of plant material, was separated into the following fractions: needles, coarse tree litter, dwarf shrub litter, the dead parts of mosses and lichens, and herb and grass litter. The results of detailed chemical analyses on the litter components are published elsewhere (*see* Hilli *et al*. 2008). The results for the total L layer, obtained as the sum of the individual litter components, are utilized in this paper. The F layer consisted of brown or dark brown fragments of dead mosses and partly decomposed litter from the trees and

**Table 1**. Site characteristics of the mesic and the sub-xeric plots in south boreal (Tammela, Punkaharju, Juupajoki) and north boreal (Kivalo, Sodankylä, Pallasjärvi) zones.

| Stand | Lat. (N) | Elevation (m a.s.l.) | Annual temp. sum (d.d.) | Precip.[1] (mm) | Precip. (mm) in 2002[2] | Stand age (yrs)* | Site index ($H_{100}$) (m) | Growing season length (days) in 2002 | Thickness (cm) of layer | | Dry weight (g m$^{-2}$) of L layer (mean ± S.D.) | Dry weight (g m$^{-2}$) of F layer (mean ± S.D.) | Dry weight (g m$^{-2}$) of H layer (mean ± S.D.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | F | H | | | |
| **Mesic** | | | | | | | | | | | | | |
| Tammela | 61°48´ | 88 | 1562 | 526 | 247 | 70 | 34.7 | 163 | 3.2 | 5.3 | 310 ± 162 | 1644 ± 651 | 4228 ± 1675 |
| Punkaharju | 61°48´ | 88 | 1524 | 526 | 129 | 70 | 34.7 | 162 | 3.3 | 5.3 | 535 ± 368 | 2398 ± 1574 | 4760 ± 1669 |
| Juupajoki | 61°51´ | 177 | 1510 | 629 | 228 | 80 | 28.0 | 152 | 3.3 | 5.3 | 291 ± 211 | 114 ± 77 | 4582 ± 4101 |
| Kivalo | 66°20´ | 252 | 1073 | 585 | 268 | 70 | 17.3 | 146 | 3.5 | 3.4 | 336 ± 95 | 2587 ± 1285 | 3172 ± 2573 |
| Sodankylä | 67°42´ | 240 | 980** | 500** | N/A | 96 | 13.4 | 114** | 3.8 | 3.8 | 290 ± 113 | 1301 ± 440 | 3684 ± 2335 |
| Pallasjärvi | 67°60´ | 300 | 889 | 545 | 254 | 140 | 10.0 | 146 | 1.5 | 4.7 | 349 ± 122 | 307 ± 194 | 3313 ± 1396 |
| **Sub-xeric** | | | | | | | | | | | | | |
| Tammela | 60°37´ | 120 | 1562 | 619 | 247 | 60 | 25.5 | 163 | 3.1 | 4.7 | 507 ± 179 | 1643 ± 533 | 4393 ± 2129 |
| Punkaharju | 61°46´ | 99 | 1524 | 526 | 129 | 80 | 26.0 | 162 | 3.1 | 3.7 | 482 ± 168 | 1334 ± 625 | 2162 ± 1255 |
| Juupajoki | 61°52´ | 154 | 1507 | 629 | 228 | 80 | 23.6 | 152 | 2.4 | 4.5 | 454 ± 157 | 1259 ± 605 | 7661 ± 4717 |
| Kivalo | 66°21´ | 145 | 1073 | 587 | 268 | 55 | 17.9 | 146 | 2.6 | 3 | 337 ± 105 | 234 ± 142 | 2286 ± 1438 |
| Sodankylä | 67°0´ | 201 | 980** | 500** | N/A | 80 | 16.0 | 114** | 1.7 | 1.9 | 319 ± 100 | 888 ± 300 | 1363 ± 1015 |
| Pallasjärvi | 67°57´ | 321 | 889 | 545*** | 254 | 90 | 14.0 | 146 | 1.2 | 1.6 | 280 ± 115 | 378 ± 347 | 626 ± 394 |

* 1999.

** measurements made at Sodankylä weather station of the Finnish Meteorological Institute.

*** value from Pallasjärvi mesic site used.

[1] Mean 1996–2003.

$H_{100}$ = mean stand height (m) at 100 years old.

[2] Measured during 1 June–30 Sep.

ground vegetation. The H layer consisted of the most decomposed material, with only a few pieces of coarse tree litter. All visible roots and rhizomes were removed from the F and H layers. The fractions and F and H layers were dried (60 °C) and weighed.

The term "stock" was used in this study to express the quantity of organic matter accumulated in the different layers in the organic horizons (L, F and H) on the mesic and sub-xeric sites. The stocks of each layer were determined as follows: (1) L layer: each of the litter fractions in each 30 cm × 30 cm plot was separated and dried and weighed (g), and the stock (g $m^{-2}$) of the total L layer obtained as the sum of the individual litter components, (2) F layer: the F layer was separated from the H layer and dried and weighed (g), and the total stock estimated (g $m^{-2}$), (3) H layer: the H layer was dried and weighed (g), and the stock estimated (g $m^{-2}$).

## Chemical analyses

The dried samples were milled to pass through a 1-mm sieve. Eight composite samples, formed from 28 sub-samples from each plot, were subjected to chemical analysis of soluble substances ($n = 96$). The milled samples were analysed using a sequential extraction technique according to Ryan *et al.* (1990) into the following fractions: nonpolar extractives (NPE, waxes, fatty acids and lipids), water-soluble extractives (WSE, e.g. sugars and phenolics), acid-soluble fraction (AS, e.g. cellulose), and acid-insoluble residue (AIR). The results for NPE, AS, and AIR are published in Hilli *et al.* (2008), and the results of the detailed analyses of the WSE fraction presented here. In the extraction procedure, 2 (± 0.010) g of dry sample was weighed into glass fibre thimbles (Gerhardt SE33A) and 120 ml of chloroform added. The samples were boiled for half an hour at 62 °C in an extraction device (Soxthern 2000). The thimbles were dried overnight at 50 °C and weighed. The difference in weight before and after extraction was taken as NPE. Water-soluble compounds: the residue was then extracted with 120 ml of distilled water, boiled at 100 °C for one hour. The water extraction was cooled and stored in plastic bottles at –18 °C until analyzed. Chloroform extraction prior to water extraction enhances extractability, because lipids and fatty acids may prevent solubility of water-soluble compounds. We used hot water because it dissolves low molecular sugars, phenols and other extractable compounds more effectively than cold water (Cheshire 1979, Angers *et al.* 1988, Ryan *et al.* 1990, Landgraft *et al.* 2006). The water-extractable phenolic concentration was determined by the Folin-Ciocalteu method (Suominen *et al.* 2003), using commercial tannic acid (VWR, Ph Eur.) as standard (Yu and Dahlgren 2000). The concentration of water-extractable sugar was determined using (D+)glucose (Merck, Reag. Ph Eur.) as standard according to the method of Wood and Bhat (1988). WEC was determined on a total organic carbon analyser (Shimadzu TOC — 5000 A). The total WEN concentrations, which included all the forms of soluble N (i.e. $NO_3$-N, $NH_4$-N, and extractable organic N), were analyzed by flow injection analysis (FIA 5012) after oxidation of N to $NO_3$-N with alkaline potassium persulphate (Williams *et al.* 1995). The dry matter content was determined by drying sub-samples for 24 hours in an oven at 105 °C. The OM content was determined as the loss in weight on ignition in a muffle furnace at 550 °C for 2 hours. The OM content was used for calculating the results per g OM.

## Data analysis

The results were expressed as concentrations per organic matter (mg $g^{-1}$ OM) and the stock per unit soil area (g $m^{-2}$). The Kolmogorov-Smirnov test and normality plots were used to test normality, and the homogeneity of the variances with Levene's test. In the case of unequal variances, square root transformations were used. Concentrations and stocks of water-extractable compounds down the organic layer profile (L, F, H) were analysed using a repeated measures ANOVA, with the layer as a within-subject factor, and plot, site type, and climatic zone as between-subject factors. Plot (a random factor) was nested with the location. Pillai's trace test was used to calculate *F* approximation (within subjects factors). The correlations between water-extractable compounds and the annual temperature sum, precipitation,

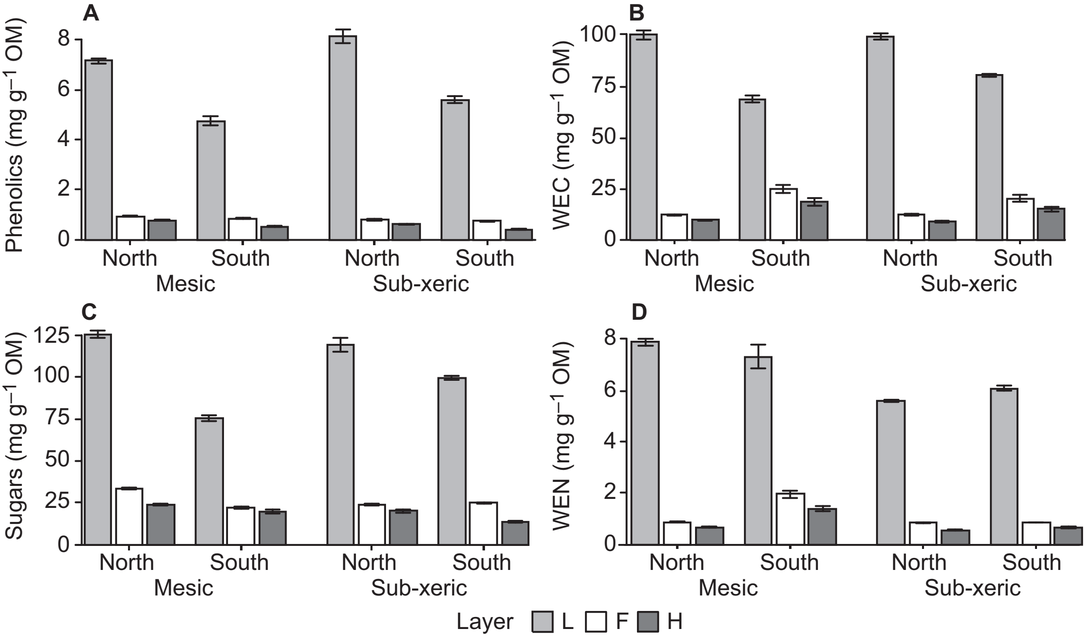

**Fig. 2**. Concentrations of (**A**) phenolics, (**B**) water-extractable carbon (WEC), (**C**) sugars, and (**D**) water-extractable nitrogen (WEN) in the L, F and H layers on the mesic and sub-xeric plots in the north and south boreal zones. The error bars indicate SE (*n* = 3).

elevation, latitude and site index were calculated with the Pearson correlation test. All analyses were carried out using SPSS 15.0 Software.

## Results

### Concentrations of water-extractable compounds in the individual organic layers

The effect of the site type and location down the vertical gradient tested with ANOVA showed that both parameters have statistically significant influences (ANOVA: $P < 0.05$ or $P < 0.001$) on all the parameters, except for the effects of site type on the concentration of sugars ($P > 0.05$), and the effect of location on concentration of WEN ($P > 0.05$). Furthermore, except for the two-way layer × location and layer × site type interactions, and three-way layer × plot(location) × site type interaction on the sugar concentrations, and the two-way location × site type interaction on the WEN and phenolic concentrations, the statistical interactions between the layer, location, site type, and plot were significant (ANOVA: $P < 0.05$ or $P < 0.001$). The concentrations of water extractable compounds decreased strongly from the L layer to the H layer (Fig. 2). The effect of location on the concentrations of different compound groups, however, varied according to the soil layer: in the L layer the concentration of phenolics, WEC and sugars were significantly higher in the north than in the south (Fig. 2A–C), whereas in the F and H layers the concentration of WEC was higher in the south (Fig. 2B). The concentration of WEN was higher in the south in all layers. Although the concentration of WEC was higher in the south, the concentrations of phenolics and sugars were significantly higher in the north, except sugars in the F layer of sub-xeric soils (Fig. 2A and C).

The concentrations of phenolics in the L layer were significantly higher on the sub-xeric than on the mesic plots, but the concentrations of phenolics in the F and H layers did not differ between the site types (Fig. 2A). In the north, there were no differences between the site types in the concentrations of WEC, whereas in the south the concentrations of WEC were significantly higher in the L layer on the sub-xeric than on the mesic plots (Fig. 2B). In the north, the concentrations of sugars were lower in all layers

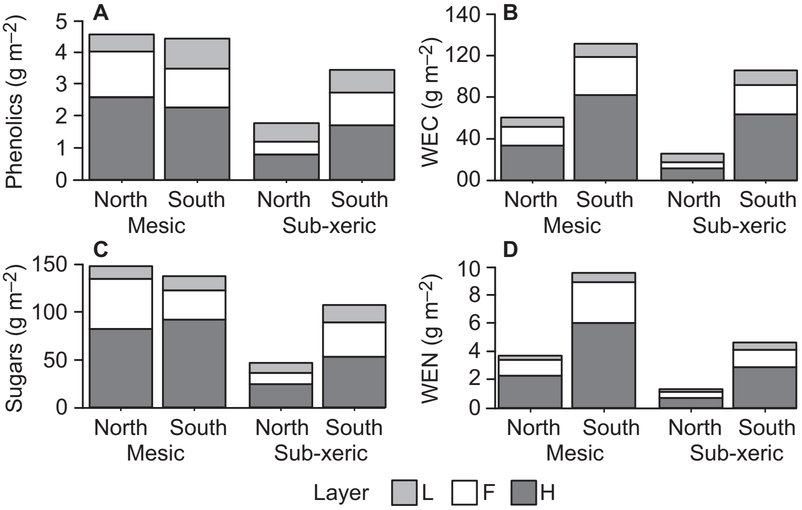

**Fig. 3**. Stocks of (**A**) phenolics, (**B**) water-extractable carbon (WEC), (**C**) sugars, and (**D**) water-extractable nitrogen (WEN) in the L, F and H layers on the mesic and sub-xeric plots in the north and south boreal zones. The error bars indicate SE ($n = 3$).

on the sub-xeric than on the mesic plots, but in the south the concentrations of sugars in the L and F layers were lower on the mesic than on the sub-xeric plots (Fig. 2C). The concentrations of WEN were higher in all layers on the mesic than on the sub-xeric plots (Fig. 2D).

## Stocks of water-extractable compounds in the individual organic layers

When calculated on an areal basis, the stocks of phenolics, sugars, WEC and WEN increased strongly down the decomposition gradient, being highest in the H layer where the total organic matter stock was also the largest (Fig. 3). The effects of soil layer, location, site type, plot, and their interactions on the stocks of water-extractable compounds were all statistically significant (ANOVA: $P < 0.001$ or $P < 0.05$), except for the two-way layer × location interaction on the stocks of phenolics, and the two-way location × site type interaction on the stocks of WEN (ANOVA: $P > 0.05$). In the F and H layers, the stocks of water-extractable compounds were significantly higher on the mesic than on the sub-xeric plots, whereas in the L layer the stocks did not differ between the site types (Fig. 3). In the F and H layers, the stocks of WEC and WEN were considerably higher in the south than in the north on both site types (Fig. 3B and D). However, the effect of climatic zone on the sugar and phenolics stocks varied between the site types: on the sub-xeric plots there were considerably more sugars and phenolics on the south than on the north boreal plots, whereas there was no significant difference on the mesic plots between the climatic zones (Fig. 3A and C).

In order to investigate the proportion of water-extractable C and N in relation to total C and N in the organic layer, we calculated the ratios for extractable *vs*. total C and N concentrations (Table 2). In the L layer the WEC/$C_{tot}$ and WEN/$N_{tot}$ ratios did not differ between the north and the south, but in the F and H layers the WEC/$C_{tot}$ ratio was significantly higher (ANOVA: $P < 0.001$) in the north. In the F and H layers, the WEN/$N_{tot}$ ratio was significantly higher on the mesic than on the sub-xeric plots (Table 2). The effects layer, location, site type, and plots, together with their interactions, on the ratios of WEC/$C_{tot}$ and WEN/$N_{tot}$ were all significant (ANOVA: $P < 0.001$ or $P < 0.05$), except for the main effect of site type, the three-way layer × location × site type interaction on WEC/$C_{tot}$, and the two way layer × location, layer × site type, and location × site type interactions on WEN/$N_{tot}$.

## Correlations between site characteristics and chemical composition of WEOM

In the L layer, the annual temperature sum and the site index correlated negatively and the precipitation during 1 June–30 Sep. 2002 correlated positively with the concentration of phenolics, sugars, and WEC (Table 3). In the F layer, the elevation correlated positively with the concentrations of sugars, and the site index correlated negatively with the total WEC/$C_{tot}$ ratio (*see* Table 3). In the H layer, the annual temperature sum correlated negatively with the concentration of phenolics, and elevation correlated positively with the concentrations of phenolics and sugars (*see* Table 3). There were no significant correlations between the long-term annual mean precipitation (1996 to 2003) and water-extractable compounds in any of the layers.

## Discussion

### Concentrations of water-extractable compounds down the vertical decomposition gradient (L, F and H layers)

In line with our hypothesis and with earlier studies conducted on forest soils (Qualls and Haines

**Table 2**. C/N, WEC/$C_{tot}$ and WEN/$N_{tot}$ in the L, F and H layers on the mesic and sub xeric site types in the north and south boreal zone. WEC = water-extractable carbon, WEN = water-extractable nitrogen (*n* = 28). Total L, F and H = average of the layer.

| | | Mesic site type | | | Sub-xeric site type | | |
|---|---|---|---|---|---|---|---|
| Site | Soil layer | C/N | WEC/$C_{tot}$ | WEN/$N_{tot}$ | C/N | WEC/$C_{tot}$ | WEN/$N_{tot}$ |
| **North** | | | | | | | |
| Pallasjärvi | L | 52.7 | 0.18 | 0.83 | 69.5 | 0.22 | 0.77 |
| Sodankylä | L | 50.3 | 0.17 | 0.71 | 69.3 | 0.19 | 0.65 |
| Kivalo | L | 45.5 | 0.25 | 0.74 | 64.4 | 0.16 | 0.72 |
| | Mean | 49.5 | 0.20 | 0.76 | 67.7 | 0.19 | 0.71 |
| **South** | | | | | | | |
| Punkaharju | L | 35.6 | 0.10 | 0.13 | 49.9 | 0.16 | 0.64 |
| Juupajoki | L | 33.9 | 0.15 | 0.75 | 58.2 | 0.16 | 0.59 |
| Tammela | L | 34.0 | 0.16 | 0.59 | 48.0 | 0.15 | 0.56 |
| | Mean | 34.5 | 0.14 | 0.49 | 52.1 | 0.15 | 0.60 |
| | **Total L** | **42.0** | **0.17** | **0.63** | **59.9** | **0.17** | **0.66** |
| **North** | | | | | | | |
| Pallasjärvi | F | 44.1 | 0.02 | 0.09 | 25.3 | 0.03 | 0.06 |
| Sodankylä | F | 39.9 | 0.03 | 0.06 | 32.8 | 0.02 | 0.07 |
| Kivalo | F | 41.3 | 0.02 | 0.06 | 40.5 | 0.02 | 0.08 |
| | Mean | 41.8 | 0.02 | 0.07 | 32.9 | 0.03 | 0.07 |
| **South** | | | | | | | |
| Punkaharju | F | 28.6 | 0.03 | 0.06 | 35.5 | 0.07 | 0.06 |
| Juupajoki | F | 27.2 | 0.03 | 0.09 | 34.2 | 0.04 | 0.07 |
| Tammela | F | 27.8 | 0.09 | 0.19 | 33.9 | 0.03 | 0.06 |
| | Mean | 27.9 | 0.05 | 0.11 | 34.5 | 0.04 | 0.06 |
| | **Total F** | **34.8** | **0.04** | **0.09** | **33.7** | **0.04** | **0.07** |
| **North** | | | | | | | |
| Pallasjärvi | H | 49.0 | 0.03 | 0.10 | 33.2 | 0.05 | 0.09 |
| Sodankylä | H | 44.1 | 0.03 | 0.06 | 37.0 | 0.02 | 0.07 |
| Kivalo | H | 54.6 | 0.03 | 0.11 | 54.1 | 0.02 | 0.07 |
| | Mean | 49.2 | 0.03 | 0.09 | 41.4 | 0.03 | 0.08 |
| **South** | | | | | | | |
| Punkaharju | H | 24.8 | 0.04 | 0.06 | 37.1 | 0.03 | 0.07 |
| Juupajoki | H | 26.5 | 0.13 | 0.10 | 32.3 | 0.03 | 0.07 |
| Tammela | H | 26.8 | 0.13 | 0.24 | 33.0 | 0.10 | 0.12 |
| | Mean | 26.1 | 0.10 | 0.13 | 34.1 | 0.05 | 0.09 |
| | **Total H** | **37.6** | **0.05** | **0.11** | **37.3** | **0.04** | **0.08** |

1992, Fröberg *et al.* 2003), we found that the concentrations of water-extractable compounds decreased down the vertical decomposition gradient. The concentrations of phenolics decreased considerably from the L to the F layer, but only slightly from the F to the H layers. A higher proportion of the soluble phenolics in the H layer consists of humified rather than plant-derived substances (Michalzik *et al.* 1999, Fröberg *et al.* 2003), and the degradability of DOC and DON decreases from the L to the H layer (Christ and David 1996, Don and Kalbitz 2005, Kiikkilä *et al.* 2006). We hypothesized that, due to the lower decomposability of phenolics in comparison to soluble sugars, the vertical gradient in the concentrations of sugars should be steeper than that of the phenolics. Our data did not support this hypothesis, which indicates that soluble phenolics do not accumulate in the F and H layers of boreal forest soils to a greater extent than soluble sugars.

The initial concentrations of nutrients and water-soluble organic compounds in plants and freshly fallen litter vary among tree species (Berg and Staaf 1980, Don and Kalbitz 2005). Plants growing on fertile soils generally have a higher concentration of N and lower concentration of phenolics (Northup *et al.* 1995, Kraus *et al.* 2004). In line with our hypothesis, we found higher concentrations of phenolics in the L layer on the sub-xeric than on the mesic plots, and negative correlation between the site index and the phenolic concentrations, which demonstrates that the inverse relationship between site fertility and the concentration of phenolics can be detected at a large geographical scale. However, although previous studies demonstrate that the concentrations and degradability of WEOM also differ in soils under different tree species (Kuiters and Denneman 1987, Howard *et al.* 1998, Suominen *et al.* 2003, Kiikkilä *et al.* 2006), we found little difference between the mesic and the sub-xeric forests in the F and H layers.

We found considerable differences in the concentrations of water-soluble compounds between the south and north boreal plots but, even more importantly, our data also showed for the first time that the changes in the WEOM concentration down the decomposition gradient varied between the south and north boreal plots. In the L layer, the WEC concentrations were higher on the north than on the south boreal plots, whereas the concentrations of WEC in the F and H layers followed an opposite trend. This shows that the chemical changes down the decomposition gradient varied between the north and south boreal zone: the reduction in the concentrations of WEC down the decomposition gradient was steeper in the north than in the south. The concentration of water-extractable compounds per OM, as expressed in our study, is determined by the decomposition rate of these compounds in relation to the non-extractable compounds. The changes in the concentration of water-extractable compounds along the decomposition continuum therefore mainly depict the proportion in which these compounds decompose in relation to the recalcitrant, non-extractable compounds.

There are a number of possible explanations for the differences down the vertical soil gradient between the south and north boreal forests. The quantity of the main types of litter in the L layer varied significantly between the south and the north boreal plots, with a higher proportion of litter from trees and mosses in the south and a higher proportion of litter from the understorey dwarf shrubs in the north (*see* Hilli *et al.* 2008). Concentrations of water-extractable compounds vary among understorey plant species (Hobbie

**Table 3**. Statistically significant Pearson correlations between the site characteristics and the concentrations of water-extractable compounds.

| | $R^2$ | $P$ |
|---|---|---|
| **L layer** | | |
| Annual temp. sum × phenolics | –0.683 | 0.05 |
| Annual temp. sum × sugars | –0.786 | 0.01 |
| Annual temp. sum × WEC | –0.681 | 0.05 |
| Precip. 1 June–30 Sep. × phenolics | +0.594 | 0.05 |
| Precip. 1 June–30 Sep. × sugars | +0.721 | 0.01 |
| Precip. 1 June–30 Sep. × WEC | +0.602 | 0.05 |
| Site index × phenolics | –0.604 | 0.031 |
| Site index × sugars | –0.850 | < 0.001 |
| Site index × WEC | –0.669 | < 0.001 |
| **F Layer** | | |
| Elevation × sugars | +0.617 | 0.033 |
| Site index × WEC/$C_{tot}$ | –0.701 | 0.011 |
| **H layer** | | |
| Annual temp. sum × phenolics | –0.698 | 0.01 |
| Elevation × phenolics | +0.764 | 0.004 |
| Elevation × sugars | +0.772 | 0.008 |

1996, Wardle *et al.* 2003) and, therefore, the difference between the north and the south in the vertical gradient could result from differences in the composition of the plant litter. Secondly, we suggest that differences in the decomposition and leaching processes may affect the vertical gradient in the concentrations of WEOM. The WEC concentrations are determined by the balance between the rate at which these substances are degraded or leached into deeper soil layers, and the rate at which they are released through the decomposition of larger molecules (Michalzik and Matzner 1999). DOC release from litter or soil organic matter increases with increasing temperature and moisture (Christ and David 1996, Tipping *et al.* 1999). The mean annual precipitation and temperature were higher on the south than on the north boreal plots and, therefore, a greater proportion of the water-soluble substances released from the L layer may accumulate in the F and H layers in the south than in the north. However, we did not find correlations between water-extractable compounds and the long-term (1996–2003) annual precipitation to support this hypothesis. In contrast, precipitation in the 2002 growing season correlated positively with the concentrations of WEC, phenolics, and sugars in the L layer, which suggests that higher precipitation had not caused greater leaching of water-extractable compounds from the L layer into the deeper organic layers. During the growing season 2002, precipitation was below the long-term average, and the positive correlation between the precipitation and the WEC concentration may reflect dry conditions. Further studies will be required to investigate the underlying reason for the differing gradients in water-extractable compounds in the south and in the north boreal forests.

The concentration of WEN was significantly lower in the north than in the south boreal zone in all the organic layers, which is in line with earlier findings suggesting that litter N concentrations decrease with increasing latitude (Bert *et al.* 1995, Johansson 1995). The concentration of WEN reflects the amount of N that is available for plant growth (Yu *et al.* 2002) and, therefore, our findings support the conjecture that the sites in the north are N deficient in relation to the sites in the south (Tamminen 2000). Our results for the WEC and WEN concentrations are somewhat higher than those reported in soils in other studies. Smolander *et al.* (2005) found 1.680 mg $g^{-1}$ of DOC and 0.067 mg $g^{-1}$ of DON (dissolved organic nitrogen) in the humus layer of a mesic, south boreal Norway spruce site. Preston *et al.* (2006) reported phenolic concentrations of 2.1 mg $g^{-1}$ in the litter and 1.4 mg $g^{-1}$ in the humus in Canadian Jack pine forests, and concentrations of 1.9–5.6 mg $g^{-1}$ in the L, 1.7–3.7 mg $g^{-1}$ in the F and 0.5–1.8 mg $g^{-1}$ in the H layer in black spruce forests. Hot water extraction and air drying the soil has been found to increase the extractability of WEC and WEN (Jones and Willett 2006). Therefore, the actual values of WEOM depend greatly on the sampling and extraction methods (Zsolnay 1996, 2003), the concentrations being higher in hot-water than in cold-water extraction.

## The role of individual organic layers as pools for water-soluble organic compounds

Although the concentrations of water-extractable compounds decreased from the L to the H layer, the total stocks of WEC and WEN increased from the L to the F layer, and from the F to the H layer. This is due to the increasing size of the organic matter stocks in the organic layers. Our data demonstrate for the first time that, in order to understand the pools of different carbon substrates, and therefore their potential contribution to the WEC and WEN leaching into the deeper soil layers, it is essential to investigate the organic fractions on an areal basis. The L layer is considered to be an important source of WEC leaching from soils (Qualls and Haines 1992, Chapman *et al.* 2001, Park *et al.* 2002), but our study demonstrates that, if the water-extractable compounds are expressed as stocks, the significance of the L layer is smaller than that of the F and H layers. Some previous studies have suggested that DOC is mainly derived from the F and H layers (Solinger *et al.* 2001, Fröberg *et al.* 2007). In agreement with the results of earlier investigations (Smolander and Kitunen 2002, Kiikkilä *et al.* 2006), the total stocks of both extractable WEC and WEN were significantly higher on the mesic than on the sub-xeric plots, reflecting the sizes of the total C stocks in the organic layer (Hilli *et al*. 2008).

The ecological linkages between the concentrations and stocks of water-soluble substances in the organic layer and the leaching of DOC and DON in percolation water are of great importance for the total C and N cycles in boreal ecosystems, but they have rarely been investigated. Neff *et al*. (2000) found that the DOC and DON fluxes are closely related to the stocks of soil organic matter. The DOC concentrations in percolation water on mesic and sub-xeric plots in the same area during 2001–2004 did not differ between the south and the north, or between the site types (Derome *et al*. 2007b). When calculated on an areal basis, the amount of leached DOC did not differ between the north and the south on the mesic sites, but the amount of leached DOC was considerably lower in the north than in the south on the sub-xeric sites (Lindroos *et al*. 2008). The amount of leached DOC was closely related to the total C stocks: the total C stocks on the mesic plots did not differ between the north and the south, but the total C stock was significantly lower in the north on the sub-xeric sites (Hilli *et al*. 2008). This is in line with an earlier study by Fröberg *et al*. (2006), suggesting that the amount of DOC leaching from the organic horizon decreases with latitude in proportion to the total soil C stocks.

The trends in the stocks of phenolics and sugars differed considerably from the stocks of WEC and WEN. In both of these compound groups, there was an interesting interaction between the site type and the climatic zone: on the sub-xeric plots the stocks of soluble phenolics and sugars were higher in the south than in the north, whereas on the mesic plots there was an opposite trend. The higher concentrations of phenolics and sugars in the north thus outweighed the role of the higher total organic matter stocks in the south, resulting in larger stocks of phenolics and sugars in the north. As the sugar and phenolic stocks were higher in the north, there must be other groups of compounds in the WEOM, the concentrations of which are higher in the south, to explain the higher total WEC stocks in the south. We did not analyze the chemical composition of the water-extractable compounds in more detail in this investigation, and further study is therefore required in order to explain the higher WEC stocks in the south boreal forests.

## Conclusions

To our knowledge, our investigation on the concentrations and stocks of water-extractable compounds down through the soil organic layer is the first investigation to link the concentrations of water-extractable compounds with the stocks of these compounds in each of the individual organic horizons. We found that despite the concentrations of water-extractable compounds are higher in the L layer, the deeper organic layers, the F and the H layer, are more important stocks for the water-extractable compounds. Therefore, the deeper soil organic layers are likely to have a high ecological importance in the DOC output by leaching from boreal forests. Furthermore, our investigation is the first study to show that the reduction in the concentrations of water-extractable compounds from the L to the H layer is much more drastic in the north than south boreal forests.

*Acknowledgements*: We are grateful to Reijo Hautajärvi and Pekka Välikangas for organizing the pre-treatment of the samples, and to all the members of staff at the Salla Office, Rovaniemi Research Unit, Finnish Forest Research Institute, who participated in the laborious task of sorting the samples. We thank Dr. Jaana Bäck and Dr. Annamari Markkola for valuable comments on the manuscript, Dr. Maija Salemaa and Leena Hamberg for providing background information on the study plots, Petteri Muukkonen for assistance in the field work, and Raimo Pikkupeura for editing the figures. The study was carried out with co-funding provided within the framework of the EU/Forest Focus programme (Regulation (EC) No. 2152/2003). The participation of S.S. was supported by the Academy of Finland (project 108235).

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