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CARBON MANAGEMENT
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# Chemical composition controls the decomposition of organic amendments and influences the microbial community structure in agricultural soils

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**ABSTRACT**

We assessed the soil carbon sequestration potential of various organic amendments of agricultural, municipal and industrial origin and the applicability of a soil carbon model to simulate it. The chemical composition of a large number of plant residues, manures, composts, digestates and biochars was determined and selected materials were incubated in soil to assess their decomposition rates and effects on soil microbial community structure. Decomposability was strongly correlated with the initial chemical composition determined by water, ethanol and acid extraction. Fresh plant materials decomposed the fastest, roots decomposed more slowly than aboveground biomass and processing decreased the decomposability of the materials. Soil carbon model Yasso07 predicted the decomposition of the amendments relatively well, except for fresh plant litter and fiber sludge from the pulp and paper industry which decomposed considerably faster than predicted by the model. Differences in the studied materials were also reflected in the soil microbial and fungal community composition. Plant root addition to laboratory microcosms induced a different soil microbial community compared to organic materials originating from the forest industry. Typical application rates of the studied amendments result in carbon sequestration at a rate sufficient to reach the goal of the 4/1000 initiative. The results can be used to select the most efficient measures to sequester carbon in croplands and to report the effects of practices like cover crop cultivation or organic matter addition.

**KEYWORDS**
soil improvement; carbon sequestration; soil carbon model; decomposition; microbial community;Yasso07

## Introduction

There is an urgent need to improve the carbon balance of cultivated soils both from the viewpoint of climate [1] and plant productivity [2]. The decreasing trend in cropland soil organic matter observed in many regions [e.g. 3, 4] reflects the imbalance between organic matter return to the soils and losses due to decomposition, leaching and erosion. Around 35-65% of the plant biomass is removed from the field with the harvested yield (calculated based on 5), and the collection of harvest residues for bioenergy production and animal bedding further decreases the amount of organic matter returning the soil [6]. The current input of carbon as crop residues generally does not maintain the carbon stocks but leads to a decrease in the stock [7–9].

Concern for soil degradation has initiated research on the potential to sequester carbon with different soil amendments [8, 10]. Use of manure improves soil carbon balance [8, 11] but in many countries animal production and thus also manure is concentrated to certain regions and as a consequence it is not optimally used from the viewpoint of soil quality [12]. Although novel manure management options, including different processing technologies, do not increase the total carbon input to soils, they can enable application of manure-based organic amendments also outside the animal farms and regions with high animal production. Manure processing is thus one possible way to improve soil quality in a wider range of fields with the existing biomasses [13].

The balance between inputs and losses of carbon can be improved by introducing plants with extensive root system or cover crops that increase carbon input to the system [14, 15]. Roots

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contribute more to the carbon stocks than above-ground residues as their exudates provide continuous carbon supply to soil and root biomass is generally chemically more resistant in comparison to above-ground biomass [7, 16]. Any organic amendments outside the farming system, such as by-products of forest and food industry and bioenergy production, return or bring new organic matter to the food production system but their properties and thus effects in soil vary greatly depending on the nature of the biomass [10, 17].

Soil organic matter is formed from degraded crop residues and other organic material as a result of microbial and geochemical processes the main constituents being plant litter and microbial necromass [18]. The major organic compounds taking part in this process include cellulose, hemicellulose, lignin, proteins, polysaccharides, tannins, lipids, cutin, suberin, chitin and acids that have different rates of decomposition when mixed with soil. However, the variables used to describe the chemical quality of organic amendments, typically proportions of the above-mentioned compounds or the carbon to nitrogen ratio, are always simplifications. The study by [7] illustrated the significance of the chemical quality of the carbon input: when the input to a long-term experiment was adjusted using a factor describing the chemical quality, it greatly improved the linear dependence between the change in the soil carbon stock and the carbon input. Processing of organic materials prior to field application e.g. by composting, anaerobic digestion or pyrolysis leaves more recalcitrant residues for the soil organisms to decompose. The persistence of organic amendments and its significance for soil carbon stocks have been studied e.g. for sewage sludges [19], cover crops [15], biochar [17, 20] and manure [21].

The current soil carbon models usually simulate humus formation based on the chemical quality of carbon input to soil and environmental variables [22–24]. However, the development of the models is based on the most common types of carbon input, such as crop residues and manure, and thus their results for the field application of more recalcitrant materials are generally not verified with field results. Future models will likely be less simplified and will include a more complex understanding of the continuum of organic compound transformations and more variables like the microbial diversity or the protective capacity of soils [25, 26].

As the role of cultivated soils in climate policies grows, also the methods to report the changes in soil carbon stocks will need to be improved. This requires improved understanding of the decomposition processes of crop residues and different substances added to cultivated soils as well as valid input data for the models used in estimating the effects of cultivation and soil management on cropland carbon stocks. Long-term experiments provide such data but only for the currently most common types of amendments.

The aims of this study were to assess the potential to increase soil carbon stocks by various organic amendments of agricultural, municipal and industrial origin, such as plant materials, manure, compost, digestate and biochar, and to find out to what extent the initial chemical composition of the amendments explains their resistance in soil and how they affect the microbial decomposer community. Furthermore, the data obtained from the set of experiments was used to evaluate the ability of Yasso07 soil carbon model to predict the decomposition of soil amendments.

## Materials and methods

### *Chemical fractionation*

The studied materials were selected to represent organic amendments originating from agriculture (plant materials such as cover crops, manure as such and after different processing), municipalities (composted sewage sludge), water protection (composted common reed) and wood industry (composted sludge, fiber sludge, biochars) (Table 1). Pretreatment of the amendments is also described in Table 1 as some of the amendments included additional materials such as peat. Samples were shredded and sieved through a 1 mm size sieve. Carbon and nitrogen contents of the samples were analysed using a dry combustion instrument (LECO, St Joseph, MI, USA) and were used to calculate the carbon to nitrogen ratios. Dry matter content of the samples was determined by drying one gram of each material overnight at 105 °C. Ash content was determined using the loss on ignition method (550 °C for 4 h). Prior to weighting, the samples were cooled in an exicator.

A method modified from [27] was used for determining the chemical composition of the organic amendments. Half a gram of each material (air dry; two replicates) was weighed into 30 ml centrifuge tubes and 20 ml ethanol was added. The samples were placed in an ultrasonic bath for 45 min followed by centrifugation (2500 rpm for

**Table 1.** **Mean (±standard deviation) of the chemical quality as acid (A), water (W) and ethanol (E) soluble and non-soluble (N) fractions, ash, carbon and nitrogen contents ($n = 2$) . Materials that were selected for detailed analysis (litterbag experiment and microcosm incubation) are highlighted in bold. Asterisk (*) depicts the materials, which were analyzed for microbial communities.**

| Material | Description | A (%) | W (%) | E (%) | N (%) | Ash (%) | Carbon (%) | Nitrogen (%) |
|---|---|---|---|---|---|---|---|---|
| **Clover shoots** | Red clover | 52.8 ± 0.4 | 31.1 ± 0.1 | 9.0 ± 0.9 | 7.1 ± 0.6 | 12.8 ± 0.2 | 41.40 | 3.05 |
| **Clover roots*** | Red clover | 59.2 ± 0.2 | 12.3 ± 1.3 | 8.4 ± 1.2 | 20.2 ± 0.3 | 16.6 ± 0.2 | 38.28 | 2.06 |
| Ryegrass shoots | Italian ryegrass | 58.3 ± 0.6 | 23.3 ± 0.0 | 7.5 ± 0.5 | 11.0 ± 0.1 | 17.3 ± 1.0 | 37.46 | 2.16 |
| Ryegrass roots | Italian ryegrass | 62.3 ± 0.5 | 8.7 ± 0.9 | 3.6 ± 0.5 | 25.4 ± 0.9 | 29.3 ± 1.4 | 33.30 | 0.94 |
| Straw | Barley | 67.6 ± 0.3 | 8.9 ± 0.2 | 5.8 ± 0.1 | 17.7 ± 0.0 | 8.9 | 43.48 | 0.62 |
| Hemp shoots | | 77.9 ± 0.6 | 3.9 ± 0.2 | 1.7 ± 0.3 | 16.5 ± 0.1 | 1.7 | 46.40 | 0.58 |
| **Common reed** | Composted for 1 yr | 61.8 ± 1.0 | 3.1 ± 0.3 | 4.6 ± 0.3 | 30.5 ± 1.6 | 13.8 ± 0.5 | 40.80 | 1.05 |
| Vegetable residues | Composted for 2 yrs, salad + other vegetables, 30% peat litter as support medium | 46.1 ± 1.0 | 5.4 ± 0.9 | 2.9 ± 0.6 | 45.6 ± 0.7 | 35.5 ± 1.0 | 30.52 | 1.31 |
| Manure, horse | Raw | 61.2 ± 0.5 | 9.2 ± 0.5 | 4.5 ± 0.6 | 25.0 ± 0.5 | 7.2 | 46.31 | 1.39 |
| **Manure, horse** | Composted, peat litter | 47.7 ± 4.1 | 6.7 ± 0.3 | 1.4 ± 0.1 | 44.2 ± 4.5 | 18.1 ± 1.9 | 39.86 | 2.11 |
| Manure, horse | Composted, straw litter | 68.4 ± 0.3 | 8.9 ± 0.2 | 2.3 ± 0.6 | 20.4 ± 0.4 | 6.7 | 44.95 | 0.92 |
| Manure, broiler | Peat litter | 53.6 ± 0.1 | 25.3 ± 0.8 | 7.9 ± 0.9 | 13.2 ± 0.2 | 14.7 | 40.39 | 4.03 |
| Manure, fox | Outdoor housing | 73.7 ± 1.8 | 8.6 ± 0.7 | 4.3 ± 0.7 | 13.4 ± 0.3 | 41.4 | 27.85 | 3.96 |
| Manure, mink | Outdoor housing | 59.6 ± 0.9 | 25.4 ± 0.6 | 7.8 ± 0.3 | 7.2 ± 0.5 | 25.7 | 34.88 | 6.09 |
| **Slurry, dairy cattle** | Raw | 52.7 ± 0.4 | 13.1 ± 0.1 | 6.6 ± 0.3 | 27.5 ± 0.8 | 19.6 ± 0.3 | 39.90 | 2.37 |
| **Slurry, dairy cattle** | Digested, co-digestion with grass silage (10% of feed w.w.) | 46.5 ± 1.1 | 14.0 ± 0.7 | 4.7 ± 0.0 | 34.8 ± 0.3 | 22.5 ± 0.1 | 38.04 | 2.27 |
| Slurry, dairy cattle | Digested and separated solid fraction | 62.4 ± 0.1 | 4.6 ± 0.3 | 2.4 ± 0.3 | 30.6 ± 0.1 | 9.3 ± 0.3 | 42.92 | 1.33 |
| Slurry, pig | Raw | 51.9 ± 0.3 | 17.0 ± 0.1 | 13.5 ± 0.3 | 17.5 ± 0.0 | 25.5 ± 0.1 | 38.65 | 3.36 |
| Slurry, mixed | Digested and separated solid fraction, co-digestion with industrial side streams (25:75 w.w.) | 31.3 ± 0.0 | 4.5 ± 0.3 | 5.8 ± 0.7 | 58.4 ± 0.9 | 65.7 ± 0.0 | 17.29 | 1.87 |
| Sewage sludge | Raw | 64.1 ± 1.0 | 4.7 ± 0.2 | 4.6 ± 0.3 | 26.5 ± 0.4 | 49.3 ± 0.0 | 25.69 | 3.35 |
| **Sewage sludge** | Composted, 30% peat litter as support medium | 61.8 ± 0.5 | 4.9 ± 0.5 | 2.3 ± 0.0 | 31.1 ± 1.1 | 41.8 ± 0.5 | 27.16 | 2.39 |
| Sewage sludge | Digested | 61.1 ± 0.5 | 2.3 ± 0.3 | 4.1 ± 0.3 | 32.6 ± 0.4 | 47.0 ± 0.1 | 27.98 | 2.90 |
| **Fiber sludge*** | Side stream of wood industry | 82.7 ± 1.6 | 3.0 ± 0.6 | 1.3 ± 1.3 | 13.0 ± 0.3 | 31.6 ± 0.2 | 33.31 | 0.04 |
| Pulp mill sludge | Lime-stabilized | 62.5 ± 0.0 | 4.6 ± 1.1 | 2.0 ± 0.7 | 30.9 ± 0.4 | 36.2 ± 0.2 | 32.55 | 0.95 |
| **Pulp mill sludge*** | Composted | 60.1 ± 4.1 | 4.0 ± 0.4 | 2.7 ± 0.4 | 33.1 ± 4.9 | 18.4 ± 0.2 | 39.45 | 0.92 |
| **Pine bark biochar** | Slow pyrolysis at 375 °C | 2.0 ± 0.0 | 0.4 ± 0.0 | 0.1 ± 0.0 | 97.5 ± 0.0 | 3.8 ± 0.1 | 70.81 | 0.43 |
| Willow biochar | Hydrothermally carbonised at 260 °C | 1.0 ± 0.4 | 0.4 ± 0.3 | 25.7 ± 0.4 | 72.9 ± 0.4 | 0.7 ± 0.1 | 70.37 | 0.64 |
| Spruce biochar | Torrefaction at 280 °C | 61.0 ± 0.6 | 2.2 ± 0.1 | 1.1 ± 0.3 | 35.7 ± 0.2 | 0.6 ± 0.1 | 51.63 | 0.07 |
| Straw biochar | Barley, pyrolysed at 460 °C | 5.2 ± 0.4 | 4.3 ± 0.4 | 3.5 ± 0.3 | 87.0 ± 1.1 | 23.5 | 66.78 | 0.93 |
| Broiler manure biochar | Slow pyrolysis at 460 °C | 17.7 ± 1.1 | 5.8 ± 0.3 | 2.6 ± 0.7 | 74.0 ± 0.0 | 34.9 | | |
| Fox manure biochar | Slow pyrolysis at 350 °C | 54.5 ± 1.0 | 2.9 ± 0.5 | 2.4 ± 0.2 | 40.2 ± 0.3 | 58.5 | | |
| Fox manure biochar | Slow pyrolysis at 450 °C | 57.1 ± 1.2 | 3.2 ± 0.3 | 1.2 ± 0.3 | 38.4 ± 1.3 | 68.1 | | |
| Mink manure biochar | Slow pyrolysis at 350 °C | 39.6 ± 0.0 | 4.8 ± 0.0 | 2.1 ± 0.4 | 53.5 ± 0.5 | 44.4 | | |
| Mink manure biochar | Slow pyrolysis at 450 °C | 34.5 ± 1.2 | 4.0 ± 0.2 | 1.4 ± 0.1 | 60.1 ± 1.2 | 51.8 | | |

10 min). The supernatant was removed with pipette and the treatment was repeated with 20 ml ethanol and 45 min sonication. Then the samples were moved to crucibles with integral glass sintered disc (grade 4 porosity) and the samples were rinsed with 30 ml ethanol using pressure assisted filtration. Samples were dried overnight at 105 °C and weighted. The weight loss represented the ethanol soluble (E) fraction. Thereafter the remaining fraction of samples was moved into 30 ml centrifuge tubes. The tube was filled with 20 ml ultrapure water and sonicated for 90 min. The samples were filtered as described above with the exception that they were rinsed with 30 ml of hot ultrapure water. The samples were dried overnight at 105 °C and weighted and the weight loss was taken as the water soluble fraction (W).

For the acid extraction, a subsample of 0.3 g was taken from each sample and moved into a 30 ml centrifuge tube with a screw cap. Further, 3 ml of 72% sulfuric acid was added and the samples were hydrolyzed in ultrasonic bath for 60 min. The samples were moved to autoclavable reagent bottles using 80 ml of ultrapure water and the hydrolysis was continued by placing the bottles into an autoclave (121 °C/1.3 bar) for 60 min. The solid fraction of each sample was separated from the liquid using sintered glasses with pressure assisted filtration. Samples were rinsed three times using 10 ml ultrapure water, dried at 105 C

overnight and weighted. The loss was taken as the acid soluble fraction (A).

Chemical composition of each material was calculated based on the mass loss due to ethanol, water and acid extraction. The results were corrected with the ash content of the sample.

In order to study the dependence of the decomposition on the initial chemical composition of the organic amendments, the A, W, E and N fractions were converted to scaled chemical quality (CQ) as follows:

$$CQ = (w_A \times \alpha_A) + (w_W \times \alpha_W) + (w_E \times \alpha_E) + (w_N \times \alpha_N)$$

where $w_A$, $w_W$, $w_E$ and $w_N$ indicate the mass proportions of the A, W, E and N fractions, respectively, and $\alpha$ indicates the decomposition rate of each fraction as defined in Yasso07 soil carbon model (see chapter 2.4). The four mass fractions sum up to one.

### Microcosm incubation

Soil for the microcosm incubation was collected from the Kotkanoja long-term field experiment of Natural Resources Institute Luke [28] located in Jokioinen in southern Finland (N 60.82°, E 23.51°). According to the World Reference Base for Soil Resources (WRB) the soil type is Protovertic Luvisol [29]. The study material was collected from the topmost 10 cm layer of an annually ploughed plot. Sand, silt and clay contents of the soil were 4.7%, 30.5% and 64.8%, respectively. Carbon content of the soil was 2.9% and pH 6.3.

Field moist soil was air-dried and sieved through a 2 mm mesh sieve. Incubation trials were prepared by mixing 30 g of soil and 1 g of each organic amendment in addition to a control with no added biomass. Three replicates of each amendment type and control were prepared. The incubation was conducted in 120 ml glass flasks at constant temperature (21 °C) and moisture conditions. The flasks were closed with perforated parafilm. Water content was set to 40% of the maximum water holding capacity of the soil and was kept constant by weighting samples weekly and adding deionized water with a pipette. Maximum water holding capacity was determined by saturating the soil for two hours in the funnel covered with filter paper following the one hour drainage and weighting.

Decomposition of the organic amendments was monitored by measuring the formation of $CO_2$ in the headspace of the flasks. Gas samples were taken 14 times during the 42-day microcosm incubation. The flasks were closed tightly with rubber septa and 1 ml of the headspace air was sampled with a syringe equipped with a needle in 1, 3 and 5 h after closing the flasks. The gas samples were analyzed for $CO_2$ concentration with the Agilent 7890 A gas chromatograph equipped with a Gilson autosampler [30]. The production rate of $CO_2$ (g $h^{-1}$) was calculated from the increase in gas concentration over time using linear regression. Finally, the carbon loss of the control samples was subtracted from those of the other treatments and the formation of $CO_2$ was converted to dry matter loss.

At the end of the incubation DNA was extracted with the NucleoSpin® Soil kit (Macherey-Nagel GmbH & Co. KG) from 0.4 g freeze dried soil according to the instruction manual with the exceptions that after adding the SL1 buffer the samples were homogenised with a FastPrep™-24 Instrument (MP Biomedicals) at 4 m/s for 30 s, then ultrasonicated for 1 h at +50 °C and further shaken with a Vortex Genie 2 (Scientific Industries) for 15 min full speed. DNA was extracted only from the following treatments: soil only control, red clover roots, fiber sludge and composted pulp mill sludge. Soil pH was measured from the same treatments using 3 ml of freeze dried soil in a soil:water suspension (1:5, v/v) according to ISO10390 (Soil quality – determination of pH) and using a pH meter.

Amplicon sequencing (Illumina MiSeq v3 600 cycles, PE 2 × 300 bp + dual index (8 bp)) was done at the Institute of Genomics of University of Tartu, Estonia. For bacteria, the 16S V4 region of the 16S SSU rRNA-gene was targeted using primers 515 F and 806 R [31, 32]. For fungi the internal transcribed spacer 2 (ITS2) region was targeted with primers gITS7 [33] and ITS4 [34].

Sequence assembly, pre-processing, chimera filtering and clustering steps were conducted with the PipeCraft 1.0 pipeline [35]. PipeCraft utilizes several implemented tools of, e.g. mothur v1.36.1 [36], vsearch v1.11.1 (github.com/torognes/vsearch; 37), and CD-HIT v4.6 [38]. Raw sequence reads were processed according to the manual with modifications for demultiplexed sequence data. In brief, assembly of paired end reads and initial quality filtering was conducted with vsearch according to default parameters except for trunc qual which was 20 for fungi and 10 for bacteria. On average one third of the raw reads were filtered out after

the assembly. Chimera filtering was performed for the reoriented reads by using vsearch de novo filtering with parameters: annotation 0.97 and abskew 2; and for ITS both reference based filtering was used with Unite ITS2 ref v7.1 as database. Also, primers and primers artefacts were filtered out from sequences at this step. In addition, fungal ITS2 region was extracted from reads with ITSx [39]. At the next step, sequence reads were clustered and OTU (operational taxonomic unit) table created with CD-hit with parameters: threshold 0.97 and min size 2. At the last step, bacterial OTUs were taxonomically annotated by searching representative sequences with BLAST by using reference 16S rRNA (SILVA_123_SSURef_Nr99_tax_silva.fasta) obtained from SILVA [41, 42]. For fungi ITS2 database (sh_genral_release_dynamic_01.12.2018.fasta) from UNITE [43] was used. After the first quality filtering steps, raw sequence data for bacteria consisted of 849533 reads clustering into 8602 OTUs. For fungi 604691 reads clustered into 1597 OTUs.

Second quality filtering was done based on the results of the sequence alignments; we filtered out bacterial and fungal OTUs that had e-values higher than e-25, identity less than 80% (bacteria) and 75% (fungi) with the database match. OTUs that had affiliation other than bacteria or fungi, or less than 5 reads were removed from the data. Furthermore, bacterial OTUs with same GenBank accession number and fungal OTUs referring to the exact same species hypothesis [44] were consolidated together. These quality filtering steps further removed singletons, rare sequences and non-target OTUs resulting in 813744 reads that affiliated to 3844 bacterial OTUs, and 509361 fungal reads that were further consolidated based on SH codes to 517 fungal OTUs/SHs. The library size varied for bacteria from 23421 reads to 58421 reads with a median of 32703. For fungi libraries varied from 13700 to 28766 reads with a mean of 21223 reads. Raw sequence data is deposited to the sequence read archive (SRA) of NCBI/EMBL database, in BioProject PRJNA609913 with the accession numbers SAMN14262759-SAMN14262806.

The sequence read data was normalized with GMPR [45]. Thereafter the amendment effect to the bacterial and fungal community compositions was analyzed with permutational multivariate analysis of variance using distance matrices with *adonis* function from vegan 2.5-5 package [46] R 3.5.2 [47]. To visualise the spatial relatedness of different microbial groups the data was plotted in three dimensional non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity matrix *metaMDS* function, and fitted the pH to the ordination with *envfit* function both from vegan 2.5-5 package. Treatment differences of bacterial and fungal OTUs were counted from log-transformed counts with *aov* function and post hoc tests using TukeyHSD.

### Litterbag experiment

The decomposition of the materials used in the microcosm experiment was also studied using litterbags buried in clay soil (Jokioinen;N 60.82°, E 23.49°). The litterbags (10 × 10 cm) were made of polyester fabric (1 mm mesh), and filled with 5 g of each material and dried at 60 °C. For each material, 20 litterbags were prepared with the exception of clover shoots and roots for which there was material sufficient for only 16 bags. The site was divided in three blocks and the litterbags were randomly placed to the blocks at the depth of 10 cm in October 2016. Two of the blocks included four litterbags for each organic material and those were collected from the soil in April and July 2017. To assure sufficient sample for the analysis after a 1-year decomposition period in October 2017, the number of litterbags in the third block was 8 for both clover shoots and roots and 12 for other materials.

The collected litterbags were first air-dried and then the material was carefully removed from the polyester bags and analyzed for mass loss. Due to the 1 mm mesh size, a variable amount of surrounding soil was incorporated into the bags. The mixture of residue and soil from the bags was ground and about 0.5 g of the ground sample was taken for the loss on ignition (LOI) analysis. The samples were incinerated at 550 °C for 5 h in a high temperature muffle furnace. This enables calculating the content of organic matter in the samples as the ignition leaves the mineral part of the soil as ash while organic matter is lost. The results from the separate LOI analysis from the original material, and the surrounding soil samples, were used in determining ash free dry weight. Organic matter loss of the materials for all three collection dates was expressed as a proportion of ash free dry weight of the initial dry weight.

Organic matter loss was modelled as a function of climate scaled time as there was great seasonal variation in climate during the experiment. Climate scaled time was calculated as a cumulative sum of the monthly decomposition coefficients (k) as

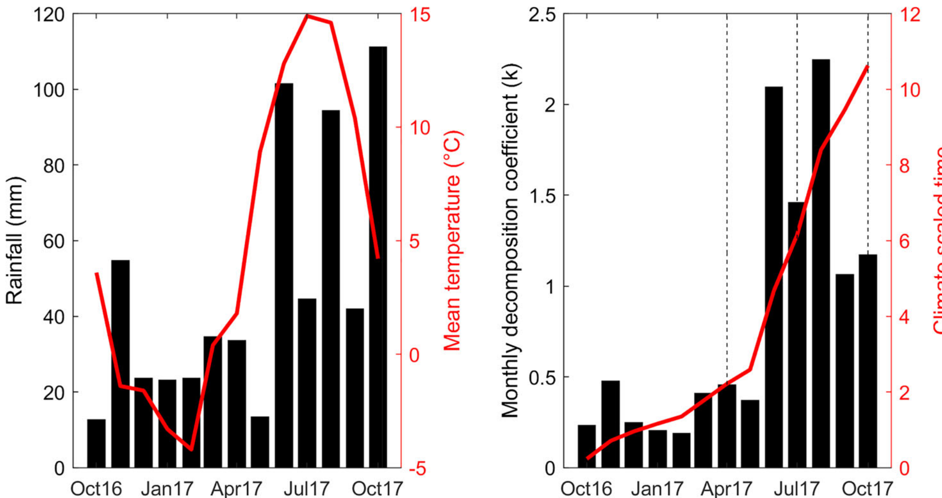


**Figure 1.** **Mean monthly temperature (curve) and rainfall (bars) from October 2016 to October 2017 (left panel). Climate scaled time (right panel) was calculated as a cumulative sum of monthly decomposition coefficients (k). The three dashed vertical lines in the right panel indicate the collection dates of the litterbags in the litterbag experiment.**

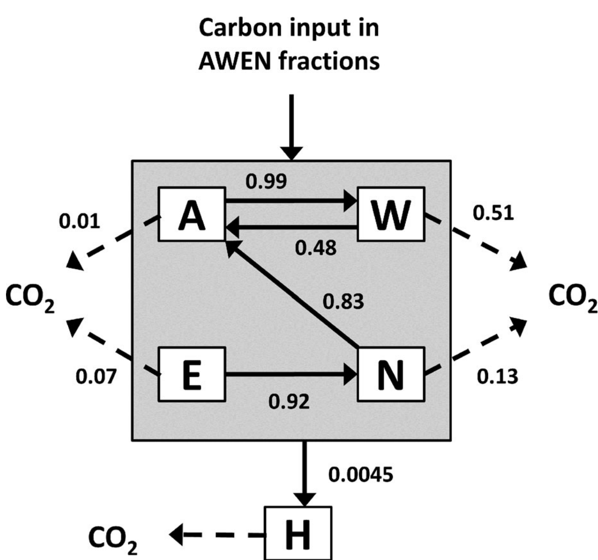


**Figure 2.** **Flow chart of Yasso07 soil carbon model. The boxes represent soil carbon as acid (A), water (W) and ethanol (E) soluble, non-soluble (N) and humus (H) fractions. The solid arrows indicate mass flow between compartments, the dashed arrows mass flow to the atmosphere and the numbers the mass flow fraction.**

described in Yasso07 model (see chapter 2.4, Figures 1 and 2). Temperature and rainfall data were taken from monthly 1 × 1 km gridded data (Finnish Meteorological Institute) by selecting the nearest grid point to the study site.

Decomposition of the organic amendments was modelled using an exponential decay function of the following form:

$$E(Y_{ijk}) = (1 - b_j)\exp(a_j t_k) + b_j$$

where $E(Y_{ijk})$ is the average organic matter proportion for the i-th litterbag of organic amendment j at the k-th time, $t_k$ is climate scaled time, $b_j$ is the asymptote of organic amendment j as t goes to infinity, and $a_j$ (constrained to be less than zero) is the rate of exponential decay which describes how quickly the process decays from the initial value to the asymptote. Due to higher variation in the observations and absence of an asymptote, the data of fiber sludge were modelled using a simpler function:

$$E(Y_{ik}) = \exp(a t_k)$$

In the models, organic matter proportions were assumed to be distributed according to a beta distribution which is a common distribution for proportions [48]. The parameters of the models were estimated using the method of maximum likelihood and standard errors of the estimates were obtained by the delta method [49]. The data included seven outlying values whose influence on the results was examined by fitting the models with and without the outliers. The modelling was implemented by the NLMIXED procedure of the SAS/STAT software (version 14.2; 50].

### *Modelling decomposition with Yasso07 model*

Yasso07 is a dynamic soil carbon model in which soil carbon is divided in five different pools: acid (A), water (W) and ethanol (E) soluble, non-soluble (N) and humus (H) pools (24, Figure 2). Carbon input is given to the model divided to AWEN fractions as well. They roughly represent the content of cellulose (A), sugars (W), waxes (E) and lignin (N) in the residues. Decomposition rates of the pools range in three orders of magnitude being the highest for the water-soluble fraction and lowest for humus. Decomposition results not only in $CO_2$ emissions to the atmosphere but also in mass flow between compartments.

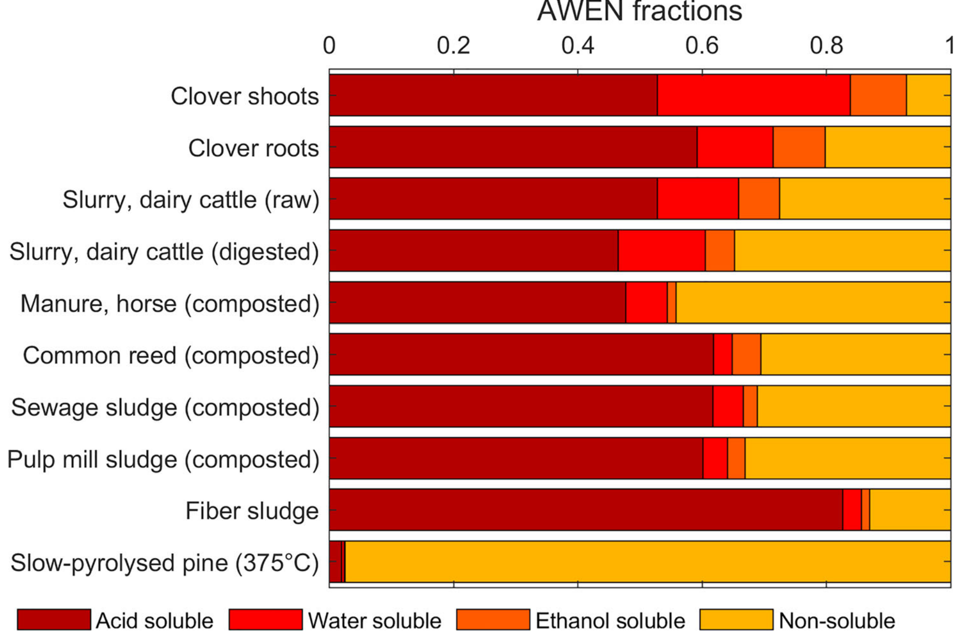


**Figure 3. Chemical quality of the materials selected for detailed analysis.**

Mean annual temperature and rainfall control the monthly decomposition rate ($R_i$) as follows:

$R_i = \alpha_i \times k = \alpha_i \times \exp(0.096 \times MT - 0.0014 \times MT^2)*(1-\exp(-1.21 \times PR \times 12))$,where $\alpha_i$ is decomposition rate of A, W, E, N and H pools ($\alpha_A = 0.73$, $\alpha_W = 5.8$, $\alpha_E = 0.29$ and $\alpha_N = 0.031$), k is the monthly decomposition coefficient, MT is the mean monthly temperature (°C) and PR is the monthly rainfall (mm).

Decomposition during the litterbag incubation of the 10 materials selected for detailed analysis was modelled with monthly timesteps with Yasso07 model using the chemical composition data from Table 1 and 1 × 1 km gridded weather data (Finnish Meteorological Institute; Figure 2). Initial mass of the materials was assumed to be equal to one and therefore the modelling results represent the remaining share of the organic matter in the end of the experiment.

## Results

### *Chemical composition of the soil amendments*

Carbon to nitrogen ratio of the soil amendments studied ranged from 6 to 833 with that of fiber sludge from the wood industry having the highest ratio (Table 1). Biochars were among the materials with high carbon to nitrogen ratios and the materials with high nitrogen content, such as manures, had the lowest values. Ash content of the materials varied from 1 to 66%.

With the exception of most biochars, the acid soluble fraction was the main component of the studied materials (Table 1). The acid soluble fraction ranged from 1% in the biochar made of willow to nearly 83% in fiber sludge. The water soluble fraction varied from close to zero in the biochars to almost 31% in clover shoots. The fraction soluble to ethanol was 26% at the highest (willow biochar). The non-soluble fraction ranged from zero to 100%.

The materials that were selected for detailed analysis also represented a variety of chemical qualities (Figure 3, Table 1). The slow-pyrolysed biochar consisted almost entirely of the non-soluble fraction (97%). The highest shares of the easily decaying water and ethanol solubles were found in fresh plant litter and dairy cattle slurry. Digestion of the cattle slurry affected the AWEN fractions slightly by increasing the share of non-soluble fraction. However, the difference is not only due to digestion but also the addition of grass silage to the slurry during processing (10% of feed w.w.).

### *Decomposition of the soil amendments*

The proportion of the remaining organic matter after the incubation with soil in microcosm conditions ranged between 31% and 100% depending on the material (Figure 4). Fresh plant litter and especially its above-ground parts lost the highest proportion of organic matter during the incubation whereas there was practically no detectable organic matter loss with pyrolysed pine bark, composted sewage sludge and composted horse manure. Digestion increased the persistence of dairy cattle slurry.

Resistant proportions of organic matter of the organic amendments studied varied between 10%

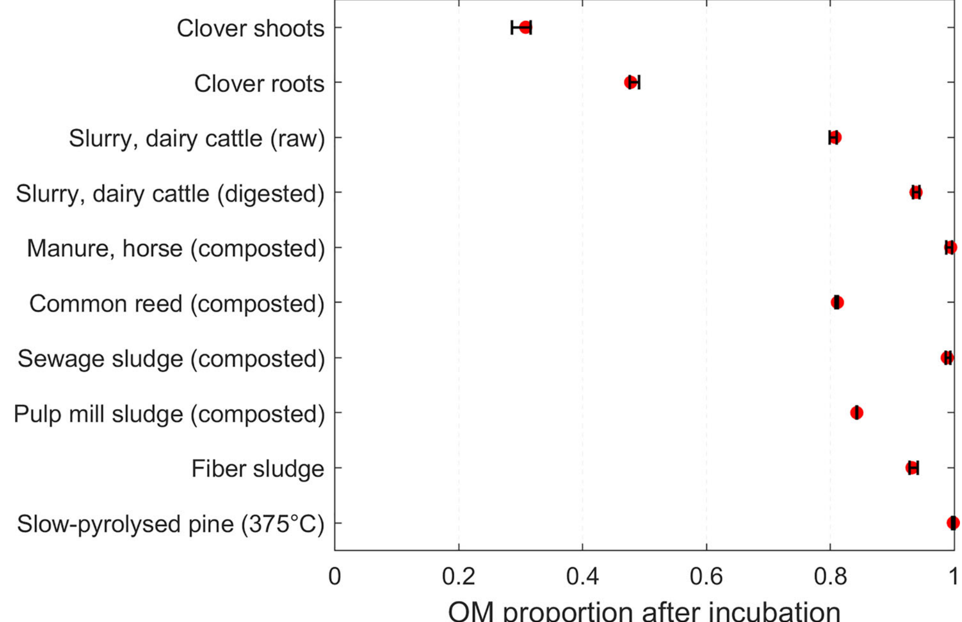


**Figure 4.** **Organic matter (OM) proportion after 6-week incubation with soil in the laboratory. Bars indicate minimum and maximum values and the circles the median value for three replicates.**

**Table 2.** **Estimates for the rate of decay and proportion of resistant organic matter (asymptote) in the models fitted to the data from the litter bag experiment without seven outliers (see Figure 5).**

| Material | n | Rate of decay | SE | Resistant proportion | SE |
|---|---|---|---|---|---|
| 1. Clover shoot | 16 | −0.65 | 0.04 | 0.10 | 0.01 |
| 2. Clover root | 16 | −0.49 | 0.05 | 0.32 | 0.01 |
| 3. Cattle slurry, raw | 17 | −0.32 | 0.04 | 0.49 | 0.02 |
| 4. Cattle slurry, digested | 18 | −0.36 | 0.07 | 0.75 | 0.01 |
| 5. Manure, horse (composted) | 18 | −1.82 | 1.39 | 0.87 | 0.01 |
| 6. Common reed | 20 | −0.04 | 0.03 | 0.25 | 0.54 |
| 7. Sewage sludge, composted | 20 | −0.64 | 0.17 | 0.84 | 0.01 |
| 8. Pulp mill sludge, composted | 20 | −0.15 | 0.03 | 0.47 | 0.05 |
| 9. Fiber sludge[a] | 17 | −0.09 | 0.01 | – | – |
| 10. Slow-pyrolyzed pine (375°)[b] | 20 | – | – | 1.00 | – |

SE = standard error; n = number of observations.
[a]The data of fiber sludge were modelled separately from the data of the other organic amendments due to higher variation in the observations and absence of asymptote.
[b]The data of the slow-pyrolysed pine were not modelled as the material did not decompose during the experiment.

and 100% in the litterbags buried in soil for one year (Table 2, Figure 5). The most resistant material was the slow-pyrolysed pine for which no mass loss was detected during the litterbag experiment. Horse manure and sewage sludge both composted with peat bedding, and digested slurry were the next most resistant of the studied materials. Digested cattle slurry was thus more resistant than raw slurry. Common reed harvested from the lakeside and composted for soil improvement decomposed slowly to the asymptote which caused a large standard error (uncertainty) in its estimated resistant proportion (Table 2). The lowest persistent proportions of organic matter were found with clover shoots and roots of which the shoots decomposed clearly faster than the roots. The variation among replicates was relatively low except for a few outliers in the case of cattle slurries, composted horse manure and fiber sludge. Ignoring the outliers, however, had a minor effect on the shape of the estimated exponential decay functions (Figure 5).

Yasso07 model predicted relatively well the decomposition of the resistant materials whereas decomposition of the easily decaying materials, fresh plant litter and fiber sludge, was underestimated (Figure 5).

### *Dependency of the proportion of resistant organic matter on the initial chemical quality of the amendment*

The degree of consistency in the rank orders of the resistant proportions of the organic amendments based on the litterbag experiment and their initial AWEN composition was high (Figure 6a). Only the common reed (6) showed notably lower resistance against decomposition in the

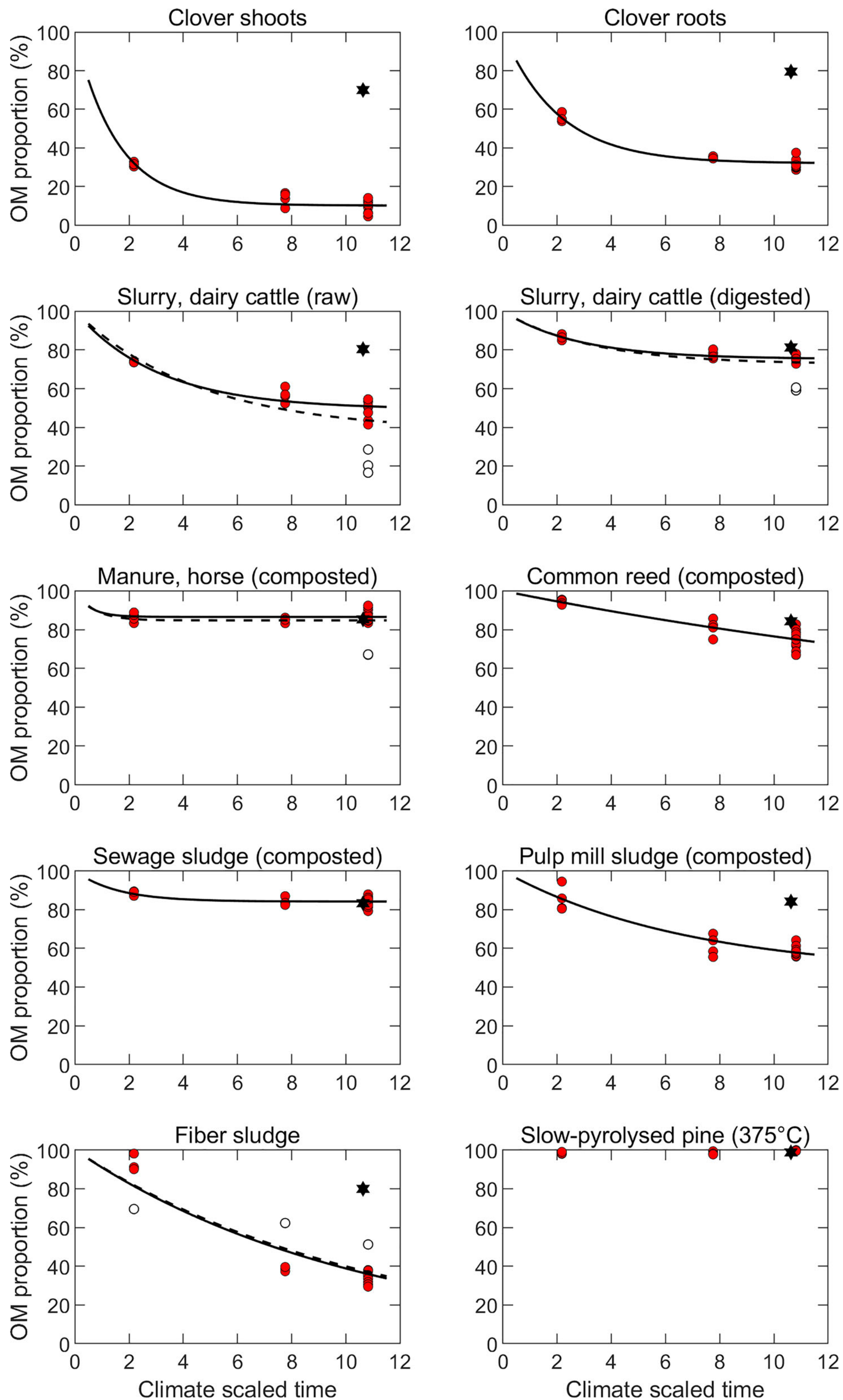


**Figure 5.** **Measured amount of organic matter (OM) of the initial amount in litter bags (circles) and decomposition according to exponential decay models fitted to the data with (dashed line) and without (solid line) outlying values (open circles). Modelled OM content in the end of the experiment using Yasso07 model is marked with a hexagram. Climate scaled time in the X-axis is defined as in Figure 1.**

litterbag experiment than expected based on its AWEN composition (see 2.1 Chemical fractionation). In comparison to AWEN composition, the carbon to nitrogen ratios and resistant proportions were less consistent (Figure 6b). The rank order of the resistant proportions of the organic amendments was fairly similar in both microcosm and litterbag experiments (Figure 6c). The exceptions were the resistant proportions of common reed (6) and pulp mill sludge (8) which were also most inaccurately estimated in the litterbag experiment.

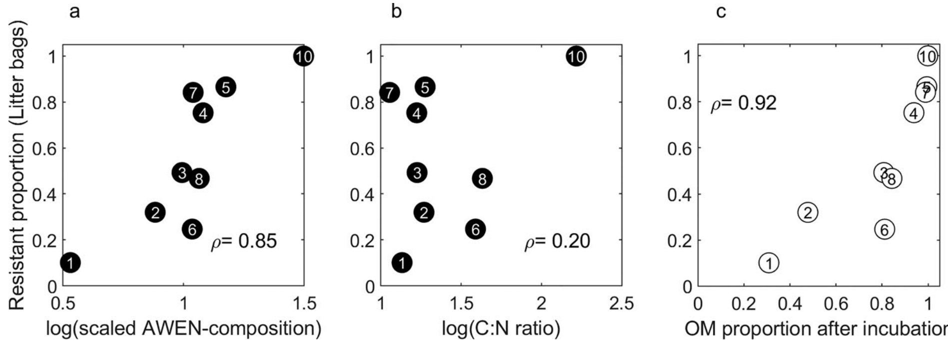


**Figure 6. Pairwise associations between the resistant proportion of organic amendments in the litterbag experiment and the chemical quality as log-transformed scaled chemical quality (CQ) (a), log C:N ratio (b) and OM proportion after the laboratory incubation (c). Numbers inside the symbols indicate the material as presented in Table 2. Spearman rank correlation coefficient (ρ) is also shown.**

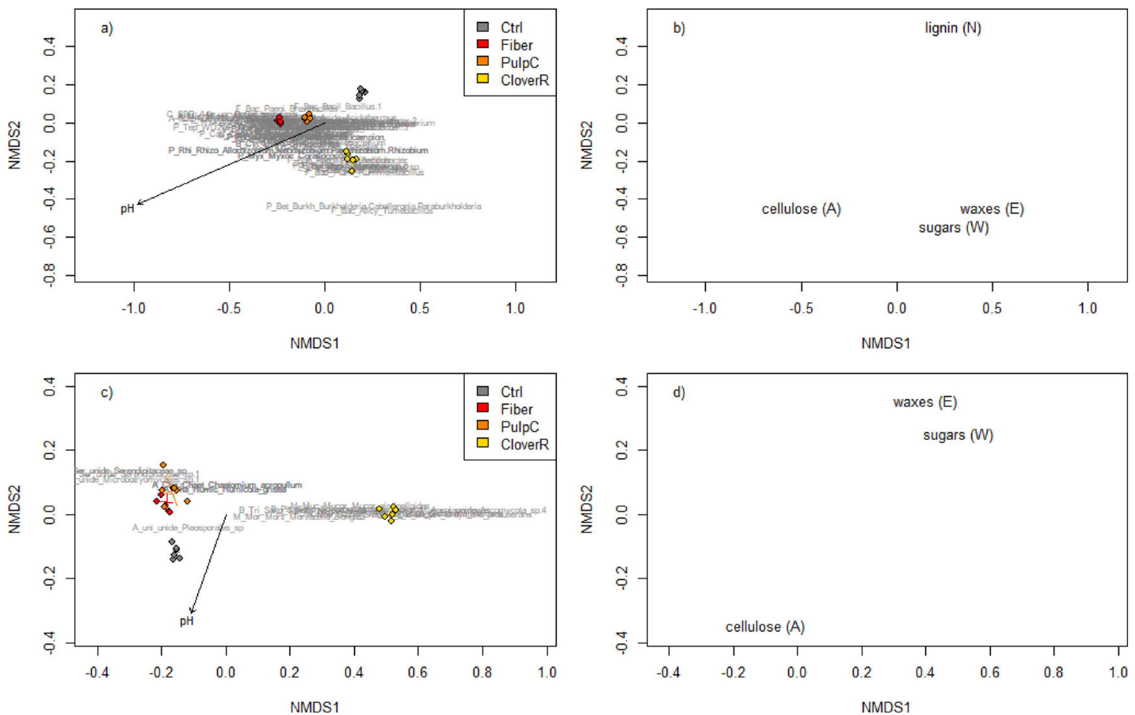


**Figure 7. 1st and 2nd axes of a 3 D NMDS of a) bacterial and c) fungal OTUs after 42 days microcosm incubation of the control soil (Ctrl) and soil amended with red clover roots (CloverR), fiber sludge (Fiber), and composted pulp mill sludge (PulpC). Arrows show the direction of increasing pH. Focal points of chemical quality based on AWEN fractions of the original amendments are visualized in the same ordination than bacteria b) and fungi d).**

## *Potential of organic amendments to modify the soil microbial community*

Bacterial diversities ranged from 1890 to 2187 operational taxonomic units (OTUs). The composted pulp mill sludge treatment had the highest number of bacterial OTUs but it differed significantly only from the clover root treatment which had the lowest diversity. Organic amendments induced a significant change in the bacterial communities and explained 69% of the variation in bacterial OTU composition. The NMDS ordination shows how the treatments group the bacterial community into four distinct groups (Figure 7a). The NMDS1-axis separates the forestry based fiber and the pulp mill sludge amendments from the control and the clover root treatments. The second NMDS axis formed the separation between the control and the clover root treatment. Over 600 bacterial OTUs were significantly varying between the organic amendments and thus contributing to the separation. The AWEN fractions contribute to both NMDS axes (Figure 7b). The higher sugar and wax content of roots compared to forest industry sidestream products distinguishes the bacterial communities. The forestry products were also characterized by higher cellulose and lignin contents. pH increased along NMDS1 and characterised the forest side stream amendments. pH in the control microcosms was 5.1 and the treatments raised pH to 5.6 in red clover treatments, 6.2 in composted pulp mill sludge and 6.8 in fiber sludge.

Fungal diversities averaged between 214 and 268 OTUs being the lowest for the clover root and the highest for the control soil. The OTU number in clover root treatment was significantly lower than in control and fiber sludge (251 OTUs) treatment. All amendments significantly differed from the control. The treatments explained 75% of the variation in the fungal community. The NMDS ordination grouped the fungal communities into three different clusters having distinct communities (Figure 7c) bulking the forest industry side stream amendments together. The main contrast along NMDS1-axis was the separation of the red clover root amended treatment from the others. The NMDS2-axis separated the control from the fiber sludge and composted pulp mill sludge amended treatments. The AWEN fractions correlated to the groupings (Figure 7d).

Ten most significant indicator species of each amendment compared to the control with soil only can be found in Table 3.

### *Potential of organic amendments to increase soil organic matter content*

Based on the litterbag experiment, the studied organic amendments can increase soil organic matter content by 88-962 kg per applied 1000 kg (DM) of the material (Table 4). Biochar had the highest potential to increase soil organic matter content but also the application of composted horse manure (with peat litter) turned out to have a relatively high impact. Although the resistant proportion of composted sewage sludge is comparatively high (0.84, see Table 2), it did not increase soil organic matter content at the same rate due to its high initial ash content. The increase of composted common reed should be treated cautiously due to the uncertainty of its estimated resistant proportion (Table 2).

For some treatments, it was possible to estimate the climate impact of the application of the amendments compared to the conventional management. Cover crops bring additional carbon to the system compared to conventional annual field crop production. It is estimated that in boreal climatic conditions the biomass of Italian ryegrass, perennial grasses and clover is 2220, 1555 and 1910 kg DM ha$^{-1}$, respectively. On average the biomass of cover crop is 800 kg DM ha$^{-1}$ for shoots and 1200 kg DM ha$^{-1}$ for roots [51]. Roughly estimated, based on the dry matter biomasses with 38% carbon content and the resistant fraction of 10% for clover shoots and 32% for roots, the cultivation of cover crops increases soil carbon stock by 175 kg C ha$^{-1}$ annually (Figure 8a).

In the case of cattle slurry, a typical 28 tonnes per hectare application of slurry (8% DM) means a soil carbon stock increase by 196 kg C ha$^{-1}$. When the same slurry is first digested, 40% of the carbon is removed as biogas but the digestion increases the share of the resistant fraction from 49% to 75%. As a result, the potential of the digested slurry to sequester carbon to the soil is close to that of raw slurry (Figure 8b).

The effect of biochar in croplands can be compared to the fate of the raw material in the system where they are derived from. In the case of biochar made of wood residues, the result can thus be compared to the situation where the residues are left in the forest (Figure 8c). Based on modelling using the Yasso07 model, only 14% of the untreated residual carbon remains in forest after 100 years, whereas 100% of the biochar can be sequestered in cropland soil. Even though 46% of the mass may be lost in the pyrolysis process, the increase in soil carbon stock would be 210 kg larger with biochar compared to leaving the residues in forest. Commonly wood residues are also burned for energy returning no carbon into soil.

## Discussion

The chemical composition of organic amendments explained their decomposition rate in soil relatively well. Dependence between litter chemical composition and decomposition is well established in previous studies (e.g. 52, 53) and the results of this study are generally in agreement with them. The resistant proportion in litterbags was more clearly associated with the AWEN composition than with the carbon to nitrogen ratio that has been found to correlate negatively with mineralization of organic amendments [54]. The study by [55] showed that simple carbon to nitrogen or lignin to nitrogen ratios are only able to describe the decomposition of fresh plant biomass, whereas the same indices are unable to predict mass loss of already decomposed litter.

Most soil carbon models (e.g. Yasso07, RothC, ICBM) are based on the assumption that litter decomposition is controlled mainly by litter quality and climate. However, it is also known that other factors, such as composition of the decomposer populations and accessibility of organic matter to them have a role as well [56], and future models

**Table 3.** **The most significantly differing indicator species (10/treatment) between amended soils and soil only control. Positive log fold change (log2FC) indicate that the OTU is more frequent in soil only control and negative that it is present in the amended soil.**

| | Bacterial 16S | | | | | Fungal ITS2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Contrast** | **Phyla** | **Family** | **Genus** | **log2FC** | **padj** | **Phyla** | **Species** | **log2FC** | **padj** |
| Ctrl-CloverR | Bacteria | Micromonosporales | Arthrobacter | −2,5 | 7,3E-57 | Ascomycota | Chaetomium acropullum | −5,8 | 1,3E-51 |
| | Firmicutes | Alicyclobacillaceae | Tumebacillus | −6,6 | 1,5E-72 | Ascomycota | Humicola grisea | −4,0 | 1,9E-40 |
| | Firmicutes | Bacillaceae | Bacillus | −3,0 | 1,3E-85 | Ascomycota | Didymella protuberans | −7,1 | 2,2E-49 |
| | Firmicutes | Bacillaceae | Bacillus | −3,2 | 6,6E-56 | Ascomycota | Gibberella intricans | −6,6 | 2,0E-60 |
| | Proteobacteria | Caulobacteraceae | Caulobacter | −3,4 | 1,1E-60 | Ascomycota | Gibberella tricincta | −4,0 | 1,4E-56 |
| | Proteobacteria | Sphingomonadaceae | Sphingomonas. | −2,7 | 4,2E-54 | Ascomycota | Pleosporales sp | **2,1** | **5,8E-58** |
| | Proteobacteria | Myxococcaceae | Corallococcus | −6,1 | 5,4E-69 | Basidiomycota | Naganishia sp | −4,5 | 9,1E-53 |
| | Proteobacteria | Burkholderiaceae | Massilia | −3,9 | 1,1E-228 | Basidiomycota | Solicoccozyma fuscescens | −4,0 | 2,5E-187 |
| | Proteobacteria | Burkholderiaceae | Burkholderia-Caballeronia -Paraburkholderia | −6,8 | 2,1E-76 | Basidiomycota | Saitozyma podzolica | −2,8 | 1,9E-69 |
| | Proteobacteria | Burkholderiaceae | Massilia | −2,1 | 9,3E-67 | Mucoromycota | Mucor circinelloides | −5,5 | 3,1E-66 |
| Ctrl-Fiber | Acidobacteria | Thermoanaerobaculaceae | uncultured | −3,8 | 4,6E-83 | Ascomycota | Chaetomidium gallecicum | −2,6 | 5,6E-16 |
| | Actinobacteria | Micromonosporaceae | Allocatelliglobosispora | −8,0 | 1,0E-96 | Ascomycota | Chaetomium acropullum | −7,3 | 2,4E-80 |
| | Planctomycetes | Gemmataceae | uncultured | −2,2 | 4,9E-56 | Ascomycota | Humicola grisea | −4,4 | 1,1E-48 |
| | Proteobacteria | Caulobacteraceae | Caulobacter | −5,2 | 2,7E-145 | Basidiomycota | Serendipitaceae sp | −10,2 | 4,1E-55 |
| | Proteobacteria | Caulobacteraceae | Phenylobacterium | −7,3 | 1,6E-49 | Basidiomycota | Serendipitaceae sp.1 | −3,2 | 6,0E-16 |
| | Proteobacteria | Rhizobiaceae | Allorhizobium-Neorhizobium -Pararhizobium-Rhizobium | −5,5 | 5,3E-60 | Basidiomycota | Serendipitaceae sp.2 | −9,3 | 1,1E-15 |
| | Proteobacteria | Archangiaceae | uncultured | −2,7 | 6,0E-99 | Basidiomycota | Microbotryomycetes sp.1 | −7,6 | 1,1E-28 |
| | Proteobacteria | Myxococcaceae | Corallococcus | −6,8 | 1,4E-87 | Basidiomycota | Sebacinales sp | −7,0 | 1,6E-25 |
| | Proteobacteria | Xanthomonadaceae | Lysobacter | −4,3 | 1,5E-105 | Chytridiomycota | Rhizophlyctis rosea | −6,7 | 5,2E-21 |
| | Verrucomicrobia | Chthoniobacteraceae | Chthoniobacter | −5,2 | 2,1E-72 | unidentified | unidentified fungi sp.1 | −11,3 | 6,8E-16 |
| Ctrl-PulpC | Acidobacteria | uncultured_bacterium | Blastocatellia | −3,6 | 4,9E-05 | Ascomycota | Pseudoproboscispora sp | **2,3** | **1,6E-05** |
| | Acidobacteria | Blastocatellaceae | Stenotrophobacter | −3,4 | 6,4E-09 | Ascomycota | Aspergillus fumigatus | −7,1 | 8,1E-16 |
| | Acidobacteria | uncultured_bacterium | uncultured | −2,9 | 2,1E-32 | Ascomycota | Chaetomium acropullum | −5,8 | 1,5E-49 |
| | Acidobacteria | uncultured_bacterium | uncultured | −3,3 | 2,5E-18 | Ascomycota | Chaetomium sp | −7,9 | 1,1E-09 |
| | Actinobacteria | Micromonosporaceae | Actinoplanes | −3,8 | 6,8E-06 | Ascomycota | Chaetomium sp.1 | −5,6 | 8,0E-06 |
| | Actinobacteria | Micromonosporaceae | Actinoplanes | **2,4** | **1,3E-11** | Ascomycota | Humicola grisea | −3,8 | 1,0E-36 |
| | Actinobacteria | Micromonosporaceae | Allocatelliglobosispora | −6,6 | 4,6E-64 | Ascomycota | Cladorrhinum sp | −7,5 | 3,2E-09 |
| | Armatimonadetes | uncultured_bacterium | uncultured | −5,6 | 7,2E-09 | Ascomycota | Pezoloma ericae | −2,2 | 1,1E-10 |
| | Armatimonadetes | uncultured_bacterium | uncultured | −4,6 | 9,5E-06 | Ascomycota | Arthrobotrys elegans | −9,7 | 5,3E-11 |
| | Bacteria | Kineosporiales | Kineococcus | −3,2 | 2,5E-08 | Ascomycota | Orbiliaceae sp | −10,1 | 9,1E-08 |

**Table 4.** **Increase in soil organic matter by organic amendments, the annual application rate required to reach the goal of the 4/1000 initiative and typical application rates.**

| Material | Effect on soil organic matter content[a] (kg 1000 kg $DM^{-1}$) | Rate[b] (kg DM $ha^{-1}$) required for 4 increase | Typical application rate (kg DM $ha^{-1}$ $yr^{-1}$) |
|---|---|---|---|
| Clover shoots | 88 | 4224 | 670 |
| Clover roots | 267 | 1395 | 1240 |
| Slurry, dairy cattle (raw) | 396 | 940 | 2200 |
| Slurry, dairy cattle (digested) | 583 | 638 | 2200 |
| Manure, horse (composted) | 709 | 525 | 7400 |
| Common reed (composted) | 213 | 1749 | 7000 |
| Sewage sludge (composted) | 490 | 760 | 7500 |
| Pulp mill sludge (composted) | 381 | 976 | 2500 |
| Fiber sludge | – | – | 3400 |
| Slow-pyrolysed pine (375 °C) | 962 | 387 | – |

[a]Based on the estimated resistant proportions in the litterbag experiment (Table 2) and on the organic matter contents of the amendments (Table 1).
[b]Calculated using the mean soil carbon stock of 54 Mg C ha in the 0-15 cm layer in Finnish cropland soils [3]. Van Bemmelen factor of 1.724 was used to convert carbon to soil organic matter content.

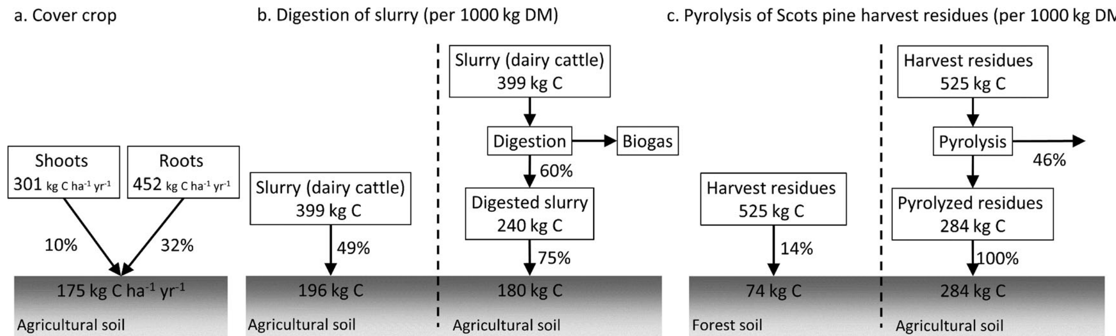


**Figure 8.** **Estimated climate impact of selected soil amendments.**

will likely include factors like microbial diversity (Louis *et al.* 2016). Recently [57] also introduced a new process-based litter decomposition model based on the NMR spectroscopy, which has been shown to characterize molecular composition of organic matter and to predict the organic matter decay more reliable than commonly used indices derived from elemental analysis or chemical extraction [55].

Based on the estimated resistant proportions, fresh plant residues decomposed the fastest of the studied organic amendments. However, roots decomposed more slowly than above-ground residues which confirmed earlier observations [7, 16]. Deep-rooted crops are seen as a way to mitigate climate change by carbon sequestration. As the data pool on the chemical quality and amounts of root biomass grows, it will enable better estimates of carbon stock changes in soil under management types with increased carbon input through roots.

Composted materials were relatively resistant in soil as the processing has already consumed the easily degradable organic matter. The resistant proportion of composts ranged from 25 to 87% which was similar to the range 20 to 70% reported in the literature [58, 59].

The resistant proportion of cattle slurry was 49% which is high compared to the carbon sequestration rates determined on the basis of long-term field experiments. Comparable manure retention coefficients reported in the literature range from 9 to 37% [21, 60]. The estimates for the carbon sequestration rates from the results of a one-year litterbag experiment should be interpreted with caution since the organic matter is subject to continuous transformations in the soil [26]. It is also to be noted that some studied amendments are mixtures of materials which have their effect on the results. For example, the horse manure and sewage sludge were mixed with peat litter in the composting process and this increases the resistant proportion in the material when used as a soil amendment. However, our aim was to study the materials as they are applied to soils and thus the additional substances were included in the analyses.

As expected, the decomposition of slow-pyrolysed biochar was too slow to be detected in a short-term incubation or litter bag experiment. Results are in line with the review by [17] indicating that 97% of biochar carbon contributes directly to long-term carbon sequestration in soil. However, the properties of biochars differ widely

depending both on the origin of the material and the production process [61, 62].

The AWEN composition of the three amendments incubated in soil was reflected in the grouping of the microbial and fungal communities. In both microbial groups, bacteria and fungi, the clover root amendment resulted in the lowest diversity. Coincidentally, clover root also had among the highest decomposition rate of the studied materials (Figure 4) and thus can reflect a different microbial succession stage compared to the other amendments.

Amendment-induced changes in bacterial communities existed but dominant functional or phylogenetic groups cannot be thoroughly discussed as more than 600 bacterial OTUs were responsible for the change. It can be said that, for instance genera *Arthrobacter*, *Bacillus*, *Burkholderia*, *Massilia* and *Sphingomonas* were more common in root amended microcosms (Table 3) and included known degraders of pesticides, pollutants and other complex organic polymers as well as antagonists against other organisms (bacteria, fungi, insects) with putative importance as biocontrolling agents and also plant growth promoters [63–67]. The amendments from wood industry increased for instance genus Actinoplanes containing known producers of antibiotics [68]. Especially the fiber treatment promoted the family Archangiaceae, and genera *Corallococcus* and *Lysobacter* [40, 69] having groups with the ability to secrete diverse secondary antimicrobial metabolites as well as the genus *Caulobacter* capable to degrade plant-derived carbon sources [70].

Within the fungal community some functional indicator species were also recognized (Table 3). *Mucor circinelloides, Solicoccozyma fuscescens, Saitozyma podzolica* as well as several potentially plant pathogenic *Gibberella* sp. were characteristic to the root treatment. *M. circinelloides* is one of the most common Mucor species typically isolated from different food products probably due to its capability to degrade cellulose [71]. *S. fuscescens* and *S. podzolica* are yeasts found worldwide and both genera are common in soil and litter [72]. As they are able to utilise carbon from cellulose they therefore contribute to dead plant biomass degradation. Amendments from wood industry increased different fungal groups compared to the root litter. Particularly fungi of Serendipitaceae and Sebacinaceae-families were abundant and distinctive to pulping residuals. Species within these groups are common root endophytes and also symbionts, and often associated with beneficial effects on host plants [73). Zoosporic fungi Chytridiomycetes such as cellulose-degrading *Rhizophlyctis* sp [74]. increased as a result of forest industry amendments. All studied amendments increased the chitinase producing fungus *Humicola grisea* [75].

As organic amendments induced clear changes in the soil microbiota, it is likely that microbes may in turn also have an influence on the soil carbon turnover of the amendments. It is notable that both the prokaryotic and eukaryotic OTU diversities resulted in very consistent patterns in the NMDS. The plant root material addition to the laboratory microcosms induced a different microbial community to evolve when compared to the treatments receiving wood industry based organic materials. These differences in the community structure can be reflected in the carbon sequestration into stable soil carbon pools. Recently, it has been estimated that soil microbial necromass can make up to 50% of the carbon stored in the soil organic carbon [76], although the influence of the microbial community structure on this process is not known. Soil microbiota also contributes indirectly to soil carbon stabilization by enhancing soil aggregate formation [77, 78]. Ref. [79] found that organic amendments with high decomposability likely act as a source of carbon for microbes and induces large but not persistent increase in aggregate stability, while cellulose rich materials have longer term effects on soil aggregates. Future research has to identify if soil microbial diversity indexes, as influenced by soil amendments, are connected to soil C sequestration and which species are possibly indicating this process.

The results in Table 4 suggest that use of most organic amendments with typical application rates can lead to carbon sequestration at a rate sufficient to reach the goal of the 4/1000 initiative. There are some signs of this in the soil survey of croplands in Finland as even coarse soils were able to sequester carbon in the animal-intensive western regions in 1998-2009, while most other soil types and regions lost carbon [3]. Interestingly, it was found in Sweden that an increase in the number of horses was related to an increase in soil carbon stocks of croplands [80]. In their study, however, it was the increased ley area rather than manure that explained this trend. The results encourage policies and measures that support the use of organic amendments in regions with a sparse animal density.

The examples in Figure 8 highlight the significance of the origin and treatment of the soil amendments. Cover crops and biochar bring additional carbon to the field ecosystem whereas in the case of manures mainly the treatment matters. The estimated rate of carbon sequestration by cover crops was about half of the global average (320 kg C ha $yr^{-1}$) reviewed by [15]. The difference is understandable as the biomass of cover crops is relatively small in boreal conditions. The results in Figure 8b encourage developing the energy use of manures as they show that the loss of carbon in the biogas process does not necessarily reduce the potential for carbon sequestration by manure. The same applies to biochar; it is likely beneficial to utilise the energy of materials first in pyrolysis instead of applying residues like straw or manure as such to soil. The climate impact of soil management is complicated as the life cycles of the materials, replaced fossil energy and effects on other land use classes should also be considered. Currently materials like forest residues or common reed are left in their natural ecosystem and thus their use on croplands only translocates carbon between ecosystems.

In this study we tested two methods of determining the resistance of organic matter, one microcosm incubation method conducted in laboratory conditions and one performed in more realistic field conditions. The rank order of the resistant proportions of the organic amendments was quite similar by both methods. However, the laboratory incubation resulted in considerably higher values for the resistant proportion suggesting that the "traditional" litterbag method cannot be replaced by the simpler one. Of course, also the litterbag method has its downsides. Although for most studied materials the one year litter bag experiment seems to be long enough, there was relatively high uncertainty in the estimated resistant proportions of composted common reed and pulp mill sludge due to the duration of the experiment and the low number of sampling over time. Further, the contact of the material in the bag with soil and especially soil macrofauna might be restricted, and part of the sample is lost from the bag for example due to leaching. These constraints should be taken into account when interpreting the results.

## Conclusions

The results indicate that decomposition of organic amendments depends on their initial chemical composition which is also reflected in the microbial community. The fractionation scheme in water, ethanol, acid and non-soluble fractions predicts the persistence of the materials relatively well and thus Yasso07 model can be used with relatively high confidence to estimate the effects of various types of carbon input on soil carbon sequestration. The study also feeds to the discussion on the most beneficial measures of carbon sequestration to be promoted by e.g. agricultural policy. The results indicated that from solely the point of view of the soil carbon stock it may be more beneficial to introduce new carbon input to agricultural systems than to change the processing of the materials already applied on fields. Application of soil amendments increases the organic matter content of the soil, but it should be noted that the climate impact of soil management is a more complicated issue as the emissions associated with all activities in the amendment's life cycle have to be considered.

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