Hindawi Publishing Corporation
Applied and Environmental Soil Science
Volume 2009, Article ID 948363, 11 pages
doi:10.1155/2009/948363

Research Article

Litter Quality of *Populus* Species as Affected by Free-Air CO_2 Enrichment and N-Fertilization

Esther Vermue, Peter Buurman, and Marcel R. Hoosbeek

Earth System Science and Climate Change, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands

Correspondence should be addressed to Esther Vermue, esther.vermue@wur.nl

Received 9 February 2009; Revised 17 August 2009; Accepted 13 October 2009

Recommended by Sabine Goldberg

The effect of elevated CO_2 and nitrogen fertilization on the molecular chemistry of litter of three *Populus* species and associated soil organic matter (SOM) was investigated by pyrolysis-gas chromatography/mass spectrometry. The results are based on 147 quantified organic compounds in 24 litter samples. Litter of *P. euramerica* was clearly different from that of *P. nigra* and *P. alba*. The latter two had higher contents of proteins, polysaccharides, and cutin/cutan, while the former had higher contents of phenols and benzofurans/pyrans. The difference between replications was at least as large as the effect of treatments, so that no systematic chemical changes were attributable to CO_2 effect or N-fertilization effect. The chemistry of SOM under the various species and treatments did not show significant changes either. The low number of available replicates that is two was clearly insufficient to overcome the effect of spatial variation on litter chemistry and detect small differences in molecular litter chemistry.

1. Introduction

The increase of atmospheric CO_2 concentration causes a fertilization effect on photoautotrophic organisms; photosynthesis rates are likely to increase and as a consequence for the terrestrial part of the carbon cycle, higher input of carbon in below and above ground biomass may result [1–5]. This additional biomass will result in increased litter input into the soil [6–8]. Soils and biomass form the largest terrestrial stocks for carbon storage, of which soils have the largest terrestrial stocks with an estimated pool of 1500 PgC, compared to an estimated biomass carbon pool of 500 PgC [7]. Soils contain carbon fractions with turnover times ranging from days to several decades and longer. For models, generally three conceptual carbon pools are defined: the detritus pool (turnover time: <10 yrs) of 300 PgC, a modified soil carbon (refractory) pool (turnover time: 10–100 yrs) of 1050 PgC, and an inert carbon pool (turnover time >1000 yrs) [7, 9]. In soils under equilibrium, the modified carbon compounds tend to form the largest pool [10]. Sequestration of soil carbon is most effective when it is allocated to the modified or inert carbon pool.

Biomass growth and subsequent C storage in soils may increase under increased atmospheric concentrations as long as other environmental factors are not limiting. One of the factors frequently suggested in literature as possibly limiting an elevated CO_2 response is nitrogen availability [11]. Feedback mechanisms between the C and N cycles might limit the fertilization effect of enhanced [CO_2], but are still not well understood [1, 11–13]. Elevated atmospheric CO_2 concentrations will not only influence the Net Primary Production (NPP), but are likely to cause changes in nitrogen-use efficiency and consequently changes in litter quality and decomposition rates [14]. Furthermore, the species composition of an ecosystem might change as a response to increased [CO_2] [12, 15].

Soil C sequestration is mainly determined by the rate of litter input to the soil system, decomposition rate, biochemical composition and SOM stabilization [12]. Decomposition is positively correlated with the litter N concentration and

negatively with C : N ratio, and lignin content. Decreases in foliar N concentration [4, 12, 16] and increases in foliar lignin concentration [12] and consequent increases in C : N ratio and lignin/N ratio have been observed as a result of elevated [CO_2] [14, 17], but trends are not consistent throughout different studies [18, 19]. Observed changes in foliar nutrient concentrations do not necessarily result in changes in litter quality due to processes like N resorption before abscission [20]. Finzi et al. [21] reports no significant change in the chemistry of leaf litter under elevated CO_2, nor was a significant effect of elevated CO_2 on the translocation of N and P from green leafs prior to senescence observed as reported by Norby et al. [12]. Changes in litter quality might cause changes in the soil microbial community. There is still controversy on the effects of elevated [CO_2] on the microbial community [22]. To make things even more complicated, different plant species show different responses to elevated atmospheric CO_2 concentrations [16, 23].

Hoosbeek et al. [5] reported that under elevated CO_2 concentrations in a Free Air CO_2 Enrichment experiment with poplars (POP-EuroFACE) more organic C is allocated to the labile organic C fraction (detritus fraction) at shallow depth (0–10 cm) than under ambient CO_2. Lichter et al. [24] and Jastrow et al. [25] report that also for the Duke Forest and Oak Ridge FACE experiments an additional sink of C is allocated to the forest floor, respectively, to the top 5 cm of mineral soil. This is further supported by Canadell et al. [1] and Cotrufo et al. [16], who found that more carbon is allocated below-ground under elevated [CO_2]. This additional organic C is allocated to the labile organic C fraction (detritus fraction). The refractory and stable fractions did not show any signs of increased carbon sequestration through elevated [CO_2] [5].

Several studies compare decomposition rates of litter produced under ambient and elevated atmospheric CO_2 concentrations (e.g., [23]). Coarse chemical characterizations such as C : N ratios and lignin contents are related to the decomposition rate [14], but they are an oversimplification of the different molecular compounds that make up the SOM. Next to an effect on the quantity of C cycling through the atmosphere-biomass-soil system, we hypothesize that FACE also affects tissue chemistry and subsequently litter quality and SOM chemistry. A change in litter quality entering the soil requires an analysis of the molecular structure of the litter and SOM. A change in litter quality (chemical composition; recalcitrance) affects respiration rates and therefore the mean residence time of SOM.

The objective of this study was to evaluate the effects of FACE, N-fertilization and species on litter and SOM chemistry. This was done by the characterization of the molecular compounds in litter and soil organic material of three different Poplar species produced under ambient and elevated CO_2 concentrations. This characterization was used to gain insight in the possible consequences for decomposition and soil carbon sequestration by changes in molecular structure. Furthermore, differences at the molecular level caused by the addition of nitrogen fertilizer are studied to assess a possible N limiting effect on the CO_2 response.

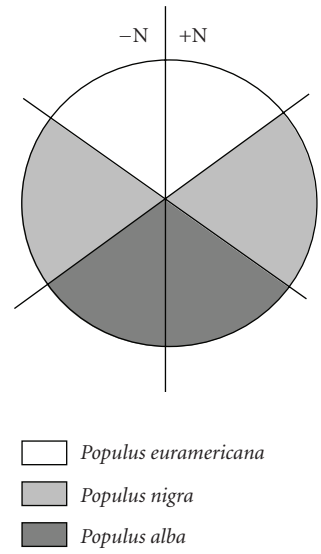

Figure 1: Layout of a POPFACE ring plot.

2. Materials and Methods

2.1. Site Description. The POP-EuroFACE experiment was established in 1999 at a site near Viterbo in central Italy (42°37′04″N, 11°80′87″E, alt. 150 m). Until 1950 the site has been under forest. It was subsequently used for agricultural production until the POP-EuroFACE site was established. The site experiences a typically Mediterranean climate with warm dry summers and humid autumns and winters. Average annual precipitation amounts to 700 mm (Xeric soil moisture regime). The soils were classified as Pachic Xerumbrepts [26].

The 9 ha POP-EuroFACE site was planted with *Populus euramericana* genotype I-214 trees, with a density of 0.5 trees per m^2. In total, six plots of ~350 m^2 each were established, 3 plots with elevated CO_2 (550 ppm) and three plots with ambient [CO_2]. Carbon enrichment was achieved by injection of pure CO_2 through laser-drilled holes in tubing mounted on six masts [27]. The FACE rings (octagons) within the FACE plots had a diameter of about 22 m. The plots were divided into two parts by a physical resin-glass barrier (1 m deep in the soil) for differential nitrogen treatments in the two halves of each plot. During the first rotation (1999–2001) no N-fertilization took place. At the end of the first rotation the trees were coppiced. In 2002, 212 kg N $ha^{-1}y^{-1}$ was applied, while in the years 2003 and 2004, 209 kg ha^{-1} y^{-1} was applied. Each half plot was divided into three sectors, where each sector was planted with one of the following genotypes: *P. x euramericana* Dode (Guinier) genotype I-214 (species 3), *P. nigra* L. (Jean Pourtet) (species 2) and *P. alba* L. genotype 2AS11 (species 1) with a density of 1 tree per m^2. Each plot thus had 6 sectors (Figure 1). The plantation was drip irrigated at a rate of 6 to 10 mm per

Table 1: Explained variance in litter sample chemistry by the four first Factors ($n = 24$).

Factor	Eigen value	% Total variance	Cumulative Eigen value	Cumulative Explained variance %
1	29.5	20.0	29.5	20.0
2	19.7	13.4	49.2	33.5
3	18.2	12.4	67.4	45.8
4	14.8	10.0	82.2	55.9

day during the growing seasons. For details of the plantation and its layout see Scarascia-Mugnozza et al. [28] and Gielen et al. [29].

Six years after establishment of the POP-EuroFACE experiment, in October 2004, litter samples were collected. A PVC ring with an inner diameter of 19 cm was placed on the forest floor and with a knife a cylindrical sample was cut. All litter within the cylinder was removed with a brush and spoon from the mineral soil and collected. A mobile refrigerator was used to transport the samples. In the laboratory, samples were dried and sieved (dry). Three size fractions were obtained by sieving (>8, 2–8, <2 mm). These size fractions largely resembled the L (almost undecomposed litter), F (recognizable, but fragmented) and H (humified) layers as observed in field. The humified (H) fraction was used for analysis. This fraction most closely reflects the chemistry of the litter that enters the soil. As the plantation was established on a slightly undulating landscape, rings 5 and 6 had, respectively, a slightly eroded top soil and a profile with some local sediment accumulation, therefore these rings were excluded from the analysis [26]. In total, 24 samples were analysed (4 rings, 6 treatments). Soil samples were taken at 0–10 cm depth from each sector (N treatment and species) within 2 control and 2 FACE rings.

2.2. Chemical Analysis. Two grams of soil were extracted with 30 mL 0.1 M NaOH. The samples were shaken for 24 hours at 20 rotations per minute under N_2 to prevent the oxidation of organic matter during extraction. The samples were then centrifuged at 3000 rpm for half an hour. The solutions were decanted and acidified to pH = 1 with approximately 3.5 mL of 1 M HCl in order to protonate the organic matter. Furthermore, 1 mL of 48% HF was added to the extracts to dissolve silicates. The extracts were then shaken for 24 hours (20 rpm) and subsequently dialyzed against demineralised water to a conductivity of <0.5 μS. This was done in cellulose membranes with a molecular cut-off of 12000 D in order to remove all excess reagents and to obtain the protonated purified organic material. After dialysis, the residues were freeze-dried and ready for pyrolysis. The litter samples were ground and freeze dried.

2.3. Pyrolysis GC/MS. For the Curie point pyrolysis, a Horizon Instruments Curie Point pyrolyser (Horizon Instruments, Heathfield, UK) was used. Litter samples were pressed onto a ferromagnetic wire and heated for 5 s to a Curie-point temperature of 600°C. The pyrolyser was connected to a Carlo-Erba GC800 gas chromatograph (Thermo Fisher Scientific, Milan, Italy) with a fused silica column (Chrompack, 25 m, 0.25 mm i.d.) coated with CP-Sil-5 (film thickness 0.40 μm) with Helium gas as a carrier. The initial temperature was 40°C, which was raised during the process at 7°C/min. The final temperature of 320°C was maintained for 20 min. The column was coupled to a Fisons MD-800 mass spectrometer (mass range m/z 45–650, cycle time 1 s). After pyrolysis, 10 samples representing all expected treatment-induced variance, were interpreted in detail from the mass spectra/pyrograms. Compounds were identified using the internal NIST library and data from literature (e.g., [30–33]). The resulting list of 291 recognized compounds was reduced to 147 compounds by deleting those that could not be identified or that occurred only once. The relative abundance of these 147 compounds with respect to the total spectrum was quantified using the peak surface of two dominant ions for each compound and calculating the percentage of this peak surface with respect to the total peak surface of all measured compounds. The compounds used represent more than 99% of the total chromatogram. The quantification method using the abundance of specific ions of a compound [34] is somewhat different from that using peak surfaces of compounds [35]. The advantage of using specific ions instead of peak surface of the compound is that peak deconvolution is hardly ever required in the former. Both methods quantify compound abundance and both suffer from the limitations that apply to quantification of pyrolysis data. While neither is a weight-percent quantification, the results of both quantifications can be used to investigate changes in chemistry, such as from one plant species to another or one treatment to another.

The most common pyrolysis compounds, their retention times and their characteristic ions are listed in Table 2. They have been grouped according to origin or structure into: *n*-alkanes and alkenes (coded by chain length), other alkanes and alcohols (Al); aromatics (Ar); fatty acids (FA); methylketones (K); lignins (Lg); sterols (Lp); poly-aromatics (PA); methyl esters (ME); phenols (Ph); polysaccharides (Ps), N-compounds (N); sulphur compounds (S) and squalene (Sq). To ensure that the samples do not show any treatment effects due to laboratory errors, samples were randomized during pyrolysis.

2.4. Factor Analysis. Factor analysis was carried out on the quantified pyrolysis results using Statistica software, version 6 (Statsoft, Inc.). Factor analysis is used to explain the variance found in the data set and to detect relationships between certain groups of variables by extracting a certain number of factors. In this study a data set was used containing all 147 identified organic matter compounds.

Table 2: Organic matter pyrolysis compounds.

Pyrolysis compound	Code	m/z	Mean r.t. (min)
$C_{10} - C_{33}$ alkanes	10 to 33	57 + 71	9.06–41.46
$C_{10} - C_{26}$ alkenes	10 : 1 to 26 : 1	55 + 69	9.26–34.66
di/trimethyl octane	Al1	57 + 71	15.32
C_{10}-alcohol	Al2	55 + 69	15.68
branched alkane	Al3	57 + 71	17.40
branched alkane	Al4	57 + 71	19.00
C12 alcohol	Al5	55 + 69	19.21
C16 alcohol	Al6	55 + 69	25.80
Alkyne	Al7	82 + 95	26.17
C18 alcohol	Al8	55 + 69	28.54
C19 alcohol	Al9	55 + 69	29.72
Benzene	Ar1	77 + 78	2.92
Toluene	Ar2	91 + 92	4.32
Ethylbenzene	Ar3	91 + 106	6.11
1,2-1,4-dimethylbenzene	Ar4	91 + 106	6.34
styrene	Ar5	78 + 104	6.80
xylene	Ar6	91 + 106	6.68
benzaldehyde	Ar7	77 + 105	8.57
C3-benzene	Ar8	105 + 120	9.83
propenyl-benzene	Ar9	117 + 118	10.11
2-hydroxy-benzaldehyde	Ar10	121 + 122	10.34
indene	Ar11	115 + 116	10.32
acetophenone	Ar12	77 + 105	10.80
1-methyl-1H-indene	Ar13	115 + 130	12.71
1-methyl-4-(1-propynyl)-benzene	Ar14	115 + 130	12.91
C3-benzene	Ar15	115 + 130	12.78
C6-benzene	Ar16	91 + 92	14.77
2-coumaranone	Ar17	78 + 134	14.79
2,3-dihydro-1H-inden-1-one	Ar18	104 + 132	15.86
C_{14}, C_{16}, C_{18} fatty acids	F14, F16, F18	60 + 73	24.40–29.90
C_{16} dioc acid	F16 : 2	60 + 73	32.37
C_{16} and C_{29} methylketones	K16, K29	58 + 59	25.56, 39.86
guaiacol	Lg1	109 + 124	11.40
4-methyl-phenol	Lg2	107 + 108	11.87
4-vinylphenol	Lg3	91 + 120	15.34
4-ethylguaiacol	Lg4	137 + 152	15.57
4-vinylguaiacol	Lg5	135 + 150	16.47
syringol	Lg6	139 + 154	17.09
4-methylsyringol	Lg7	153 + 168	18.86
trans 4-(prop-2-enyl) guaiacol	Lg8	149 + 164	19.06
4-acetylguaiacol	Lg9	151 + 166	19.81
4-vinylsyringol	Lg10	165 + 180	21.13
4-(prop-2-enyl) syringol *trans*	Lg11	194 + 91	23.45
4-acetylsyringol	Lg12	181 + 196	24.43
1-(2,6-dihydroxy-4-methoxyphenyl),3-phenyl-2-propen-1-one	Lg13	193 + 270	33.31
cholesta-4,6-dien-3-diol	Lp1	135 + 143	38.28
cholest-5-en-3-ol compound	Lp2	81 + 147	38.48
cholest-5-en-3-ol, (3. Beta.-) compound	Lp3	81 + 145	39.09
cholest-x-en-x-ol compound	Lp4	81 + 147	40.46
alkanoic acid methyl ester	ME	74 + 87	17.95
naphthalene	PA1	128	13.59

Table 2: Continued.

Pyrolysis compound	Code	m/z	Mean r.t. (min)
1/2-methylnaphthalene	PA2	141 + 142	16.11
phenol	Ph1	66 + 94	9.59
2-methylphenol	Ph2	107 + 108	11.17
dimethyl phenol	Ph3	107 + 122	13.26
ethyl phenol	Ph4	107 + 122	13.75
pristene	Pr	55 + 69	23.46
2- methylfuran	Ps1	53 + 82	2.43
1-methyl-1,3-cyclopentadiene	Ps2	79 + 80	2.77
acetic acid	Ps3	60	3.03
2-ethyl-furan	Ps4	81 + 96	3.40
2,5-dimethyl-furan	Ps5	95 + 96	3.46
3-methyl-2-cyclopenten-1-one	Ps6	67 + 96	3.59
(2H)-furan-3-one	Ps8	54 + 84	5.02
2-ethyl-5-methylfuran?	Ps9	95 + 110	4.92
2-furaldehyde	Ps10	95 + 96	5.26
2-cyclopenten-1-one?	Ps11	82 + 53	5.57
3-furaldehyde	Ps12	95 + 96	5.87
3-methyl butanoic acid	Ps13	45 + 60	7.03
2-methyl-2-cyclopenten-1-one	Ps14	67 + 96	7.22
2-acetylfuran	Ps15	95 + 110	7.74
2,3-dihydro-5-methylfuran-2-one	Ps16	55 + 98	8.30
5-methyl-2-furaldehyde	Ps17	109 + 110	8.66
dianhydrorhamnose	Ps18	113 + 128	10.32
2/7-methylbenzofuran	Ps19	131 + 132	11.47
levoglucosenone	Ps20	68 + 98	11.69
2/7-methyl benzofuran	Ps21	131 + 132	11.81
2,3,4,5-tetra-O-methyl d-glucose	Ps22	101 + 45	13.47
1,4:3,6-dianhydro-.alpha.-d-glucopyranose	Ps23	57 + 69	14.30
2,3-dihydro-benzofuran	Ps24	91 + 120	14.86
6-deoxy-3-O-methyl L-glucose	Ps25	74 + 73	17.28
3-methyl-2(3H)-benzofuranone	Ps26	91 + 148	17.59
3-methyl-2H-1-Benzopyran-2-one	Ps27	131 + 160	20.44
levoglucosan	Ps28	60 + 73	20.60
Propanenitrile?	N1	54 + 55	3.53
methyl-1H-pyrrole?	N2	80 + 81	3.88
4,5-dihydro-2,4-dimethyl-1H-imidazole	N3	69 + 98	3.97
pyridine	N4	52 + 79	4.16
pyrrole	N4a	67	4.21
1/3-ethyl-1H-pyrrole	N5	80 + 95	5.12
2-methyl-pyridine	N6	66 + 93	5.82
3-methyl-1H-pyrrole	N7	80 + 81	5.93
2-methyl-1H-pyrrole	N8	80 + 81	6.25
3-methyl-pyridine	N9	66 + 93	6.76
2,3-dimethyl-1H-pyrrole	N10	80 + 95	7.75
benzonitrile	N11	76 + 103	8.89
1-(2-pyridinyl)-ethanone	N12	79 + 78	10.15
2-methyl-benzoxazole	N13	78 + 133	12.00
benzeneacetonitrile	N14	117 + 90	12.43
indole	N15	90 + 117	16.27

Table 2: Continued.

Pyrolysis compound	Code	m/z	Mean r.t. (min)
x-methyl indole	N16	130 + 131	18.04
diketodipyrrole	N17	93 + 186	24.13
amide	N18	59 + 72	30.43
4-hydroxy-benzenesulfonic acid	S	65 + 94	3.73
squalene	Sq	69 + 81	37.05

Codes: Al: aliphatics, Ar: aromatics, F: Fatty acids, K: methylketones, Lg: lignins, Lp: lipids (sterols), ME: methylester, N: N-containing compounds, PA: polyaromatics, Ph: phenols, Pr: pristene, Ps: polysaccharides, S: S-containing products, Sq: Squalene, m/z: masses used for quantification; r.t: retention time.

For the data set, the Eigenvalues, factor loadings, and factor scores were calculated by extracting four factors. Using more factors would result in a higher explained variance, but the first factors have the highest explanatory value. The Eigenvalues represent the variance extracted by the factors. In case all variance is explained by the extracted factors, the sum of Eigenvalues is equal to the number of variables. All samples have a factor score that reflects the trends in the dataset by giving the samples a weighted combination of the original variables that were highly correlated, that is, the factor score [36]. These factor scores can be plotted in the factor space. The underlying factor loadings, that is, the contribution of each organic compound to the various factors are used to understand and explain the factor scores plot.

3. Results

The soil and litter data were analyzed separately. Carbon dioxide enrichment did not affect SOM chemistry, and therefore these results are not reported here. The effect of *Populus* litter on SOM chemistry could not be investigated because soil samples taken prior to the POP-EuroFACE experiment were not available. The results will therefore be restricted to the litter fraction.

For the factor analysis, all 24 samples with the 147 organic compounds were used. Table 1 presents the Eigen values and explained variance for the first four factors. Of the total variance, 55.9% is explained. The results are discussed according to the different treatments applied: species effect, N-fertilization effect, CO_2 treatment effect and ring (replication) effect. The discussion will be restricted to Factors 1 and 2. Plots of Factors 3 and 4 did not show the various effects more clearly.

3.1. Species Effect. The sample scores for the first two factors are presented in Figure 2, where the circles indicate the three different tree species. The majority of the samples are concentrated around the 0-axis of factor 2. All samples for species 3 have a negative score on Factor 1 and can be distinguished from species 1 and 2, that have positive scores on Factor 1 (except sample 2110). Samples of species 1 and 2 overlap on both factors, and therefore these species can not be distinguished chemically. Figure 3 presents the factor loadings of the individual organic matter compounds. Compounds having factor scores of $-0.5 < x < 0.5$ are considered to have too little explanatory value and will not be discussed in the following.

All four phenol compounds, and compounds Ar17 (2-coumaranone), Ar10 (benzaldehyde), Lg3 (4-vinylphenol), Ps19 and Ps21 (2- and 7-methylbenzofuran), Ps26 (3-methyl-2(3H)-benzofuranone, and Ps27 (3-methyl-(2H)-benzopyran-2-one) have negative scores for Factor 1 and are concentrated around the 0-axis of Factor 2. Species 3 (*Populus euramericana*) appears to have larger contents of the compounds of this cluster. Although phenols are sometimes pyrolysis products of amino acids, this appears not to be the case here because there are no nitrogen-containing compounds plotting in the same area. The benzopyran/furan compounds (Ar17, Ps19, 21, 26, 27) are generally associated with a polysaccharide origin and are probably a distinctive feature of the litter of *Populus euramericana.*

The second main cluster of organic matter compounds has positive scores on Factor 1, which corresponds to species 1 and 2 (Figures 2, 3). This cluster consists for the major part of polysaccharide and aliphatic compounds, of which the polysaccharide compounds have higher loadings. A large number of aromatic and N-containing compounds plot at the lower right. These compounds are usually pyrolysis products of amino acids/proteins. Compared to species 3, species 1 and 2 are relatively enriched in alkanes and alkenes, which are presumably derived from cutan [37], and in polysaccharides and N-compounds.

Of the polysaccharide compounds, the cellulose derived components are all located in the upper right corner of the plot. These components [Ps18 (dianhydrorhamnose), Ps20 (levoglucosenone), Ps22 (2,3,4,5-tetra-O-methyl-D-glucose), Ps25 (6-deoxy-3-O-methyl-L-glucose) and Ps28 (levoglucosan)] are obviously most abundant in samples 4110, 1210, 4210, and 3110, which have all received N-fertilization. These polysaccharide compounds plot close to the long-chain alkanes (C_{23}–C_{33}), which tend to represent relatively undegraded litter.

The N-containing compounds with enough relevance (i.e., N1, N2, N3, N4, N7, N8, N9, N10, N12, N13, N14, N17) are all located in the lower right corner of the scatterplot. They are all pyridine and pyrrole compounds and an imidazole compound. The pyrrole compounds are derived from proline (Irwin, 1982 in [38]), and the pyridine compounds are pyrolysis products of α- and β-alanine, chitin and polypeptides. The facts that these compounds plot opposite the markers of relatively fresh litter and that samples with N-fertilization do not systematically plot in

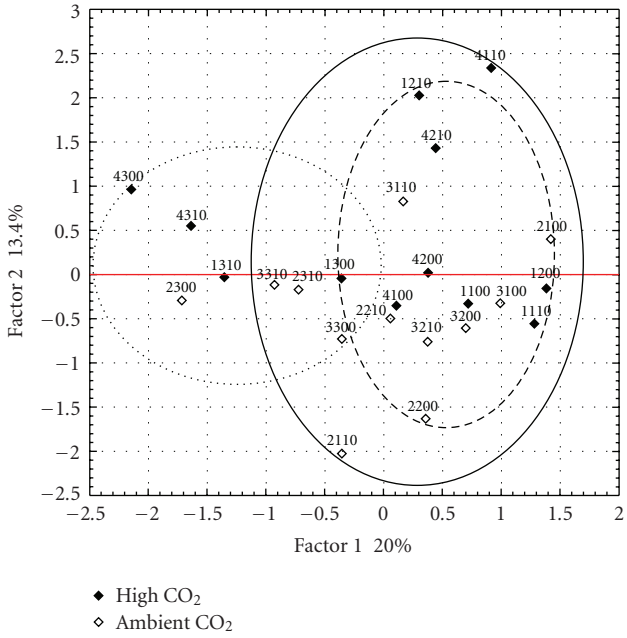

Figure 2: Species effect: Litter factor scores in F1F2 space. Sample numbers consist of four figures indicating, respectively, ring number (1 to 4), species number (1 to 3), N fertilization (0 no, 1 yes) and litter or mineral soil sample (0, 1). Solid line: species 1; dashed line: species 2; dotted line: species 3.

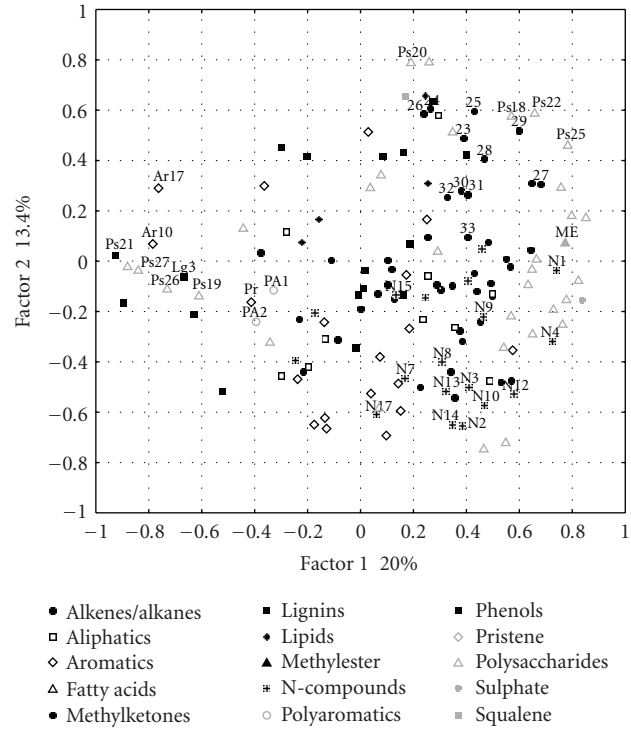

Figure 3: Factor loadings in F1F2 space.

the lower right quadrant may suggest that they represent microbial degradation products. The finest litter fraction (<2 mm; H) is also the most strongly degraded one and likely to contain products of microbial origin. Also in other data sets, these N-containing compounds represent strongly degraded SOM and therefore probably microbial products [39, 40]. Indole (N15), which is associated with fresh SOM, plots close to (0,0). This suggests that the <2 mm litter fractions of species 1 and 2 differ in degradation state, which hinders the interpretation of treatment effects.

3.2. Location Effect. The location of four different rings introduces considerable spatial variability. The replicate rings give some idea of the spatial variability present, that is, samples 4100 to 4310 are replicates of samples 1100 to 1310 and samples 2100 to 2310 are replicates of samples 3100 to 3310. Figure 4 presents the litter sample scores for the first two factors, but the different rings are indicated.

The samples of rings 2 and 4 cover the largest area in factor space and almost completely envelop those of rings 1 and 3. As seen before, most samples plot close to the zero axis of Factor 2, but for rings 2 and 4 more samples are located outside this cluster, especially samples 4110, 4210, 4300, 4310, 2300, 2100, and 2200. The samples in ring 3 show the lowest chemical variation. The samples of rings 2 and 4 show different scores for factor 2 for more than one sample of the sample group. The rings also indicate that the samples of the replicate pairs have different factor scores. The difference between replicates 1110/4110, 2110/3110 and 1300/4300 is considerable, while pairs 2310/3310, 2210/3210 and 1210/4210 show only little difference. The other replicate pairs have differences between these extremes. The difference in factor score between the samples varies both in amount and direction. As the rings were randomly positioned in the field, these differences in litter chemistry may be due to spatial soil variability as found for any field experiment. It is known from stable isotope research that differences in field circumstance, for example, hydrology, cause a shift in plant isotopic signature. Because the overall isotopic signature is a weighted average of the signatures of the constituent chemical compounds and because the signatures of the compounds are very different (e.g., proteins have much less negative $\delta^{13}C$ values than aromatics or aliphatics [41]), shifts in overall isotopic signature indicate shifts in chemical composition.

3.3. CO_2 Effect. The two circles in Figure 5 present the treatment effect due to CO_2 fertilization. The two circles show a large overlap. The samples show a spread throughout the scatterplot, but a cluster of elevated-CO_2 samples has strongly positive scores on Factor 2, while two ambient-CO_2 samples have strongly negative scores. Because the upper left corner of the Factor loadings diagram of Figure 3 does not contain any organic compounds, the projection of the high-CO_2 samples in this corner of Figure 5 should be seen as a relative depletion of the molecular compounds of the lower right corner of Figure 3, that is, the compounds that represent proteins. This suggests that elevated CO_2 may cause a decrease in such components. Figure 5, however, clearly indicates that this is not a universal effect. When samples

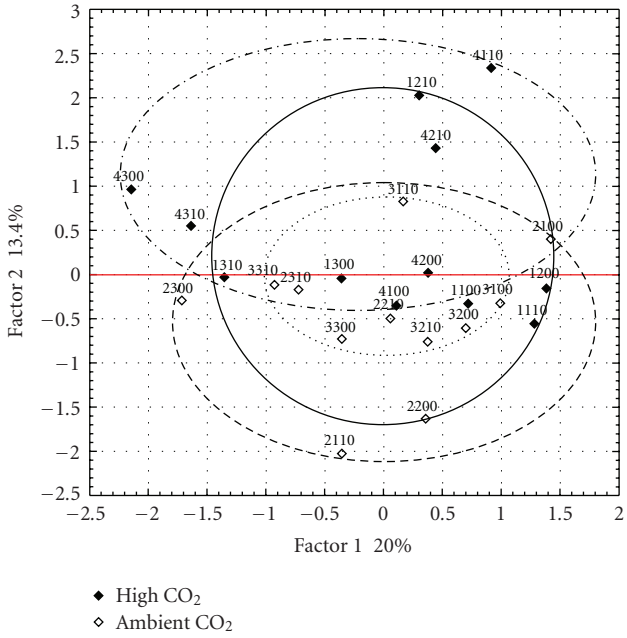

Figure 4: Location effect: litter factor scores in F1F2 space. Solid line: ring 1; dashed line: ring 2; dotted line: ring 3; dashed-dotted line: ring 4.

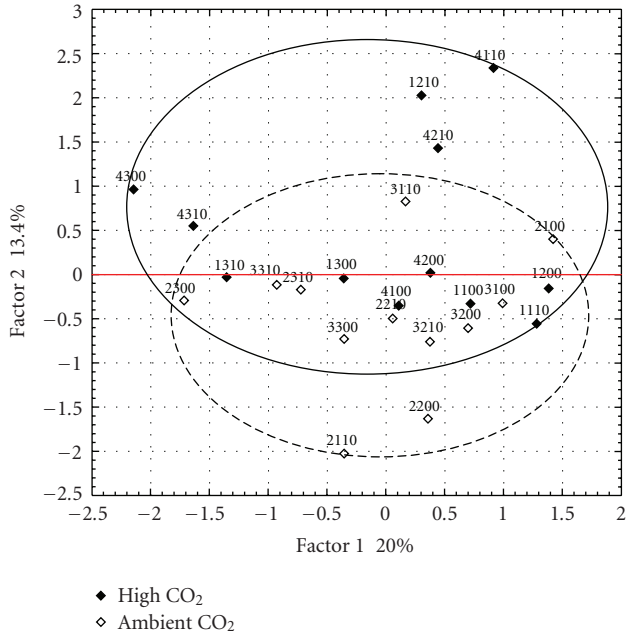

Figure 5: CO_2 effect: Litter factor scores in F1F2 space. Solid line: samples with elevated [CO_2]; dashed line: samples with ambient [CO_2].

from ring 1 and 3, as previously discussed are left out of the analysis, only samples 4200, 4100 and 2310 are part of the overlapping area. Then, elevated CO_2 samples have a positive score on Factor 2, except for sample 4100. Ambient CO_2 samples have a negative score on Factor 2, except for sample 2100. The overlap between the two treatments remains too large, and elevated CO_2 effects on litter chemistry are not evident.

3.4. N-Fertilization Effect. The effect of N-fertilization is presented in Figure 6, where the fertilized and nonfertilized samples are indicated by the solid and the dashed lines, respectively. The circles have a large overlap, indicating no systematic chemical differences between litter of fertilized and nonfertilized plots. The nonfertilized samples have a larger spread on Factor 2. Replicates with respect to N-fertilization do not plot together in factor space. If the location effect is eliminated and sample pairs within one ring are compared, no N-fertilization effect on litter chemistry is observed.

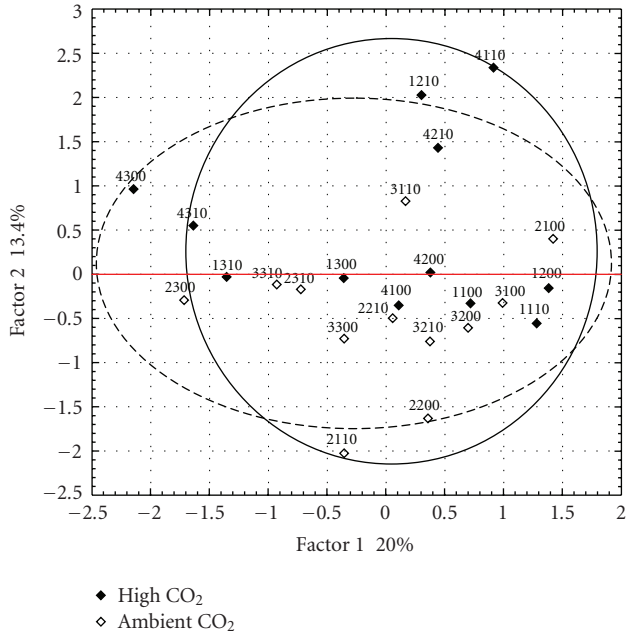

Figure 6: N-fertilization effect: Litter factor scores in F1F2 space. Solid line = samples with N-fertilization, dashed line = samples without N-fertilization.

4. Discussion

No previous study reports on the use of the pyrolysis GC/MS method to study the effect of ambient/elevated CO_2 on litter chemistry. There have, however, been studies on the effect of elevated CO_2 on litter quality and on typical pyrolysis products of poplar litter. Meier et al. [42] demonstrated that Py-GS and principal component analysis allow the distinction of woods of different poplar clones. The present study could not discriminate between leaf litter (<2 mm) of *Populus nigra* and *Populus alba*. Leaves and especially senescent (or slightly decomposed) leaves seem to be less distinctive for poplar species than wood samples. Species 3 (*Populus euramericana*) can be distinguished from the other two based on relatively low amounts of N-compounds and a relative

enrichment of phenols and benzopyrans/furans while *Populus nigra* and *Populus alba* yield more polysaccharide, aromatic, and N-compounds. For the different poplar species of the POP-EuroFACE experiment, Marinari et al. [43] found clear differences in terms of fresh leaf nutrient concentrations.

Although the effect of CO_2 fertilization on litter chemistry is not unequivocal, possibly due to the low number of replicates, ambient [CO_2] litters of species 1 and 2 appear to have a larger content of N-containing compounds and related aromatics compared to the elevated [CO_2] samples. The elevated [CO_2] samples of *P. alba* and *P. nigra* seem to have relatively high amounts of polysaccharides (cellulose products) and lignin. Cotrufo et al. , [23] did not find a similar response for the POP-EuroFACE experiment; leaf litter did not show any changes in lignin concentration due to elevated CO_2 conditions during a short term experiment of 8 months.

The larger content of N-containing compounds and related aromatics for ambient [CO_2] litters support the previously observed increase in NUE for elevated [CO_2] litters of the POP-EuroFACE experiment. Cotrufo et al. [23], found a reduction in N leaf litter concentration for the POP-EuroFACE experiment under elevated [CO_2]. Each of the three species showed a different response, with *P. euramericana* having the highest reduction. Calfapietra et al. [44] showed for the POP-EuroFACE experiment that NUE increased under elevated CO_2, in contrast to other forest FACE experiments where no increase in NUE was reported [11]. The increase in NUE was confirmed by data from the BangorFACE experiment, where NUE also increased. Both BangorFACE and POP-EuroFACE experiments are plantations on former agricultural soils where N supply is more than sufficient. Therefore the NUE can increase under elevated CO_2 when N is a nonlimiting factor, and the C/N can decrease [45].

The N supply associated with former agricultural fields is the most likely cause of N fertilization not having affected the molecular chemical composition of the litter. Neither did the combination with elevated [CO_2] induce such changes. De Graaff et al. [46] showed that for the Swiss grassland FACE project, no positive or negative feedback was observed for N mineralization of plant materials after 9 years. They suggested that agricultural soil management practices have a greater impact on the soil N cycle than CO_2 fertilization.

Decomposition rates are strongly correlated with C/N ratio. The C/N ratio of the total forest floor was not affected by FACE due to increased N immobilization under FACE [47], but decomposition rates decreased during later stages under FACE [23]. Our results, with relatively less proteins under elevated [CO_2] and more structural carbohydrates and lignin, could lower decomposition rates when these molecular structures are more resistant to degradation. Some of the N-fertilized samples had relatively undegraded fragments. Without N-fertilization SOM is decomposed to obtain N, but as N is abundantly available less decompostion is needed to obtain new N.

Enhanced sequestration of carbon in soils might mitigate climate change. Enhanced sequestration would result from both an increase in yearly litter input and a shift of litter chemistry towards more unpalatable compounds. The present research suggest that, although litter quality may differ significantly between species, effect of CO_2 and N fertilization is minor. Differences in the fresh litter must have disappeared during the initial phase of litter fragmentation and decomposition, so that litter quality changes did not play a role. Differences in SOM were largely due to the amount of input. Hoosbeek et al. , [5] indicated that under FACE the input of labile C (detritus pool) for the first 10 cm of soil was increased compared to ambient conditions. The largest input of C was found under *P. euramericana* for, respectively, 2000 and 2001; *euramericana* averaged 16 and 15% more new C in 2000 and 2001, respectively, compared to the other two species [48]. In soil, no species effect or N-fertilization effect was found for the labile C and N-fractions. The labile C was considered to consist mainly of nonstructural carbohydrates (fructose, glucose, sucrose and starch) derived from litter and root exudates. Lichter et al. [8] observed that an increase of soil C in the upper 15 cm under FACE at the Duke forest was also due to the labile C fraction while the other C fractions remained unaltered. The refractory and stable SOM fractions were not affected by CO_2 fertilization.

In the POP-EuroFACE experiment, the species effect on carbon sequestration was much larger than any other effect. Hoosbeek et al. [48] provided that the input of new C was highest under *P. euramericana* compared to the other two species due to a larger litter input, which provides an increased number of foci for microbial activity and thus nucleation centers for aggregation and resulting protection. Together with a different litter quality for *P. euramerica* as found in the present research, this may result in the long term in increased formation of micro-aggregates and thus in increased long-term stabilization of SOM [47]. The chemical differences found in litter of *P. euramericana* on one hand and *P. alba* and *P. nigra* on the other may lead to differences in C sequestration. Compared to *Populus euramericana*, *Populus alba* and *nigra* are relatively enriched in cutin, cutan (the alkane/alkene precursors), polysaccharides, and proteins, while *P. euramericana* litter is richer in phenols. These combinations of characteristics do not allow conclusions as to the relative stability of each litter type or of the resulting SOM. However, under *P. euramericana* more free micro-aggregates were formed, indicating that more newly incorporated soil C was stabilized and protected [47]. Cotrufo et al. [23] reports that the leaf litter of *P. euramericana* and *P. nigra* decomposes at lower rates than *P. alba* leaf litter. Moreover this relative stability depends on the soil characteristics as a whole. Aliphatics (and lignin) are recalcitrant when circumstances for decomposition are not ideal (low temperature, water logging, low pH, high Al), but not when soils are fertile and temperatures are high.

We conclude that FACE and N-fertilization did not affect the litter chemistry and associated SOM chemistry of the three Populus species. Initial differences between species in litter chemistry did not result in differences in decomposition.

References

[1] J. G. Canadell, L. F. Pitelka, and J. S. I. Ingram, "The effects of elevated [CO_2] on plant-soil carbon below-ground: a summary and synthesis," *Plant and Soil*, vol. 187, no. 2, pp. 391–400, 1996.

[2] W. Cheng, "Rhizosphere feedbacks in elevated CO_2," *Tree Physiology*, vol. 19, no. 4-5, pp. 313–320, 1999.

[3] W. H. Schlesinger and J. Lichter, "Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO_2," *Nature*, vol. 411, no. 6836, pp. 466–469, 2001.

[4] R. J. Norby, P. J. Hanson, E. G. O'Neill, et al., "Net primary productivity of a CO_2-enriched deciduous forest and the implications for carbon storage," *Ecological Applications*, vol. 12, no. 5, pp. 1261–1266, 2002.

[5] M. R. Hoosbeek, Y. Li, and G. E. Scarascia-Mugnozza, "Free atmospheric CO_2 enrichment (FACE) increased labile and total carbon in the mineral soil of a short rotation poplar plantation," *Plant and Soil*, vol. 281, no. 1-2, pp. 247–254, 2006.

[6] D. S. Schimel, "Terrestrial ecosystems and the carbon cycle," *Global Change Biology*, vol. 1, no. 1, pp. 77–91, 1995.

[7] IPCC, *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, New York, NY, USA, 2001.

[8] J. Lichter, S. H. Barron, C. E. Bevacqua, et al., "Soil carbon sequestration and turnover in a pine forest after six years of atmospheric CO_2 enrichment," *Ecology*, vol. 86, no. 7, pp. 1835–1847, 2005.

[9] D. S. Schimel, B. H. Braswell, E. A. Holland, et al., "Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils," *Global Biogeochemical Cycles*, vol. 8, no. 3, pp. 279–293, 1994.

[10] R. S. Swift, "Organic matter characterization," in *Methods of Soil Analysis. Part 3. Chemical Methods*, D. L. Sparks, Ed., pp. 1011–1069, Soil Science Society of America, Madison, Wis, USA, 1996.

[11] A. C. Finzi, R. J. Norby, C. Calfapietra, et al., "Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO_2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 35, pp. 14014–14019, 2007.

[12] R. J. Norby, M. F. Cotrufo, P. Ineson, E. G. O'Neill, and J. G. Canadell, "Elevated CO_2, litter chemistry, and decomposition: a synthesis," *Oecologia*, vol. 127, no. 2, pp. 153–165, 2001.

[13] K.-J. Van Groenigen, J. Six, B. A. Hungate, M.-A. de Graaff, N. van Breemen, and C. van Kessel, "Element interactions limit soil carbon storage," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 17, pp. 6571–6574, 2006.

[14] M. F. Cotrufo, P. Ineson, and A. P. Rowland, "Decomposition of tree leaf litters grown under elevated CO_2: effect of litter quality," *Plant and Soil*, vol. 163, no. 1, pp. 121–130, 1994.

[15] A. C. Finzi and W. H. Schlesinger, "Species control variation in litter decomposition in a pine forest exposed to elevated CO_2," *Global Change Biology*, vol. 8, no. 12, pp. 1217–1229, 2002.

[16] M. F. Cotrufo, P. Ineson, and A. Scott, "Elevated CO_2 reduces the nitrogen concentration of plant tissues," *Global Change Biology*, vol. 4, no. 1, pp. 43–54, 1998.

[17] P. De Angelis, K. S. Chigwerewe, and G. E. Scarascia-Mugnozza, "Litter quality and decomposition in a CO_2-enriched Mediterranean forest ecosystem," *Plant and Soil*, vol. 224, no. 1, pp. 31–41, 2000.

[18] J. S. King, K. S. Pregitzer, D. R. Zak, M. E. Kubiske, J. A. Ashby, and W. E. Holmes, "Chemistry and decomposition of litter from Populus tremuloides Michaux grown at elevated atmospheric CO_2 and varying N availability," *Global Change Biology*, vol. 7, no. 1, pp. 65–74, 2001.

[19] M. C. Hall, P. Stiling, D. C. Moon, B. G. Drake, and M. D. Hunter, "Elevated CO_2 increases the long-term decomposition rate of *Quercus myrtifolia* leaf litter," *Global Change Biology*, vol. 12, no. 3, pp. 568–577, 2006.

[20] D. L. Randlett, D. R. Zak, K. S. Pregitzer, and P. S. Curtis, "Elevated atmospheric carbon dioxide and leaf litter chemistry: influences on microbial respiration and net nitrogen mineralization," *Soil Science Society of America Journal*, vol. 60, no. 5, pp. 1571–1577, 1996.

[21] A. C. Finzi, A. S. Allen, E. H. DeLucia, D. S. Ellsworth, and W. H. Schlesinger, "Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment," *Ecology*, vol. 82, no. 2, pp. 470–484, 2001.

[22] D. R. Zak, W. E. Holmes, A. C. Finzi, R. J. Norby, and W. H. Schlesinger, "Soil nitrogen cycling under elevated CO_2: a synthesis of forest face experiments," *Ecological Applications*, vol. 13, no. 6, pp. 1508–1514, 2003.

[23] M. F. Cotrufo, P. De Angelis, and A. Polle, "Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO_2 enrichment (POPFACE)," *Global Change Biology*, vol. 11, no. 6, pp. 971–982, 2005.

[24] J. Lichter, S. A. Billings, S. E. Ziegler, et al., "Soil carbon sequestration in a pine forest after nine years of atmospheric CO_2 enrichment," *Global Change Biology*, vol. 14, no. 12, pp. 2910–2922, 2008.

[25] J. D. Jastrow, R. M. Miller, R. Matamala, et al., "Elevated atmospheric carbon dioxide increases soil carbon," *Global Change Biology*, vol. 11, no. 12, pp. 2057–2064, 2005.

[26] M. R. Hoosbeek, M. Lukac, D. van Dam, et al., "More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): cause of increased priming effect?" *Global Biogeochemical Cycles*, vol. 18, no. 1, Article ID GB1040, 7 pages, 2004.

[27] F. Miglietta, A. Peressotti, F. P. Vaccari, A. Zaldei, P. DeAngelis, and G. Scarascia-Mugnozza, "Free-air CO_2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system," *New Phytologist*, vol. 150, no. 2, pp. 465–476, 2001.

[28] G. Scarascia-Mugnozza, P. De Angelis, M. Sabatti, et al., "Global change and agro-forest ecosystems: adaptation and mitigation in a FACE experiment on a poplar plantation," *Plant Biosystems*, vol. 139, no. 3, pp. 255–264, 2005.

[29] B. Gielen, C. Calfapietra, M. Lukac, et al., "Net carbon storage in a poplar plantation (POPFACE) after three years of free-air CO_2 enrichment," *Tree Physiology*, vol. 25, no. 11, pp. 1399–1408, 2005.

[30] J. Ralph and R. D. Hatfield, "Pyrolysis-GHC-MS characterization of forage materials," *Journal of Agricultural and Food Chemistry* , vol. 39, no. 8, pp. 1426–1437, 1991.

[31] H.-R. Schulten and M. Schnitzer, "Structural studies on soil humic acids by Curie-point pyrolysis-gas chromatography/mass spectrometry," *Soil Science*, vol. 153, no. 3, pp. 205–224, 1992.

[32] P. F. Van Bergen, I. D. Bull, P. R. Poulton, and R. P. Evershed, "Organic geochemical studies of soils from the Rothamsted Classical Experiments—part I: total lipid extracts, solvent

insoluble residues and humic acids from Broadbalk Wilderness," *Organic Geochemistry*, vol. 26, no. 1-2, pp. 117–135, 1997.

[33] P. F. Van Bergen, M. B. Flannery, P. R. Poulton, and R. P. Evershed, "Organic geochemical studies of soils from the Rothamsted Classical Experiments—part II: nitrogen-containing organic matter in soil from Geescroft Wilderness," in *Nitrogen-Containing Macromolecules in the Bio-and Geosphere*, B. A. Stankiewicz and P. F. van Bergen, Eds., pp. 321–338, American Chemical Society, Washington, DC, USA, 1998.

[34] F. A. de Alcântara, P. Buurman, N. Curi, A. E. F. Neto, B. van Lagen, and E. L. Meijer, "Changes in soil organic matter composition after introduction of riparian vegetation on shores of hydroelectric reservoirs (Southeast of Brazil)," *Soil Biology and Biochemistry*, vol. 36, no. 9, pp. 1497–1508, 2004.

[35] G. Halma, D. Van Dam, J. Haverkamp, W. Windig, and H. L.C. Meuzelaar, "Characterization of an oligotrophic-eutrophic peat sequence by pyrolysis-mass spectrometry and conventional analysis methods," *Journal of Analytical and Applied Pyrolysis*, vol. 7, no. 1-2, pp. 167–183, 1984.

[36] C. M. Hoover, K. A. Magrini, and R. J. Evans, "Soil carbon content and character in an old-growth forest in northwestern Pennsylvania: a case study introducing pyrolysis molecular beam mass spectrometry (PY-MBMS)," *Environmental Pollution*, vol. 116, supplement 1, pp. S269–S275, 2002.

[37] E. W. Tegelaar, J. W. de Leeuw, and C. Saiz-Jimenez, "Possible origin of aliphatic moieties in humic substances," *The Science of the Total Environment*, vol. 81-82, no. C, pp. 1–17, 1989.

[38] H.-R. Schulten and M. Schnitzer, "The chemistry of soil organic nitrogen: a review," *Biology and Fertility of Soils*, vol. 26, no. 1, pp. 1–15, 1997.

[39] P. Buurman, K. G. J. Nierop, X. Pontevedra-Pombal, and A. Martinez Cortizas, "Molecular chemistry by pyrolysis-GC/MS of sleceted samples of the Penido vello peat deposit, Galicia, NW Spain," in *Peatlands: Evolution and Records of Environmental and Climate Change*, I. P. Martini, A. Martinez Cortizas, and W. Chesworth, Eds., pp. 217–240, Elsevier, Amsterdam, The Netherlands, 2006.

[40] P. Buurman, F. Peterse, and G. A. Martin, "Soil organic matter chemistry in allophanic soils: a pyrolysis-GC/MS study of a Costa Rican Andosol catena," *European Journal of Soil Science*, vol. 58, no. 6, pp. 1330–1347, 2007.

[41] T. W. Boutton and S. I. Yamasaki, *Mass Spectrometry of Soils*, Marcel Dekker, New York, NY, USA, 1996.

[42] D. Meier, I. Fortmann, J. Odermatt, and O. Faix, "Discrimination of genetically modified poplar clones by analytical pyrolysis-gas chromatography and principal component analysis," *Journal of Analytical and Applied Pyrolysis*, vol. 74, no. 1-2, pp. 129–137, 2005.

[43] S. Marinari, C. Calfapietra, P. De Angelis, G. E. Scarascia-Mugnozza, and S. Grego, "Impact of elevated CO_2 and nitrogen fertilization on foliar elemental composition in a short rotation poplar plantation," *Environmental Pollution*, vol. 147, no. 3, pp. 507–515, 2007.

[44] C. Calfapietra, P. De Angelis, B. Gielen, et al., "Increased nitrogen-use efficiency of a short-rotation poplar plantation in elevated CO_2 concentration," *Tree Physiology*, vol. 27, no. 8, pp. 1153–1163, 2007.

[45] M. R. Hoosbeek, M. Lukac, E. Velthorst, and D. L. Godbold, "Free atmospheric CO_2 enrichment did not affect symbiotic N_2—fixation or soil carbon content and stabilization in a mixed deciduous stand in Wales," submitted to *Global Change Biology*.

[46] M.-A. De Graaff, J. Six, H. Blum, and C. van Kessel, "Prolonged elevated atmospheric CO_2 does not affect decomposition of plant material," *Soil Biology and Biochemistry*, vol. 38, no. 1, pp. 187–190, 2006.

[47] M. R. Hoosbeek and G. E. Scarascia-Mugnozza, "Increased litter build up and soil organic matter stabilization in a poplar plantation after 6 years of atmospheric CO_2 enrichment (FACE): final results of POP-EuroFACE compared to other forest FACE experiments," *Ecosystems*, vol. 12, no. 2, pp. 220–239, 2009.

[48] M. R. Hoosbeek, J. M. Vos, E. J. Bakker, and G. E. Scarascia-Mugnozza, "Effects of free atmospheric CO_2 enrichment (FACE), N-fertilization and poplar genotype on the physical protection of carbon in the mineral soil of a polar plantation after five years," *Biogeosciences*, vol. 3, no. 4, pp. 479–487, 2006.