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Effects of free atmospheric CO₂ enrichment (FACE), N fertilization and poplar genotype on the physical protection of carbon in the mineral soil of a polar plantation after five years

M. R. Hoosbeek¹, J. M. Vos¹, E. J. Bakker², and G. E. Scarascia-Mugnozza³

¹Wageningen University, Department of Environmental Sciences, Earth System Science Group, P.O. Box 37, 6700AA Wageningen, The Netherlands

²Wageningen University, Department of Plant Sciences, Mathematical and Statistical Methods Group, P.O. Box 100, 6700AC Wageningen, The Netherlands

³University of Tuscia, Department of Forest Environment and Resources, Via S.Camillo De Lellis, 01100 Viterbo, Italy

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Abstract. Free air CO₂ enrichment (FACE) experiments in aggrading forests and plantations have demonstrated significant increases in net primary production (NPP) and C storage in forest vegetation. The extra C uptake may also be stored in forest floor litter and in forest soil. After five years of FACE treatment at the EuroFACE short rotation poplar plantation, the increase of total soil C% was larger under elevated than under ambient CO₂. However, the fate of this additional C allocated belowground remains unclear. The stability of soil organic matter is controlled by the chemical structure of the organic matter and the formation of micro-aggregates (within macro-aggregates) in which organic matter is stabilized and protected. FACE and N-fertilization treatment did not affect the micro- and macro-aggregate weight, C or N fractions obtained by wet sieving. However, *Populus euramericana* increased the small macro-aggregate and free micro-aggregate weight and C fractions. The obtained macro-aggregates were broken up in order to isolate recently formed micro-aggregates within macro-aggregates (iM-micro-aggregates). FACE increased the iM-micro-aggregate weight and C fractions, although not significantly. This study reveals that FACE did not affect the formation of aggregates. We did, however, observe a trend of increased stabilization and protection of soil C in micro-aggregates formed within macro-aggregates under FACE. Moreover, the largest effect on aggregate formation was due to differences in species, i.e. poplar genotype. *P. euramericana* increased the formation of free micro-aggregates which means that more newly in-

corporated soil C was stabilized and protected. The choice of species in a plantation, or the effect of global change on species diversity, may therefore affect the stabilization and protection of C in soils.

1 Introduction

Afforestation of agricultural land and regrowth of temperate forests constitute a large carbon (C) sink (Houghton, 2003; Houghton et al., 1998; Janssens et al., 2003). Enhanced growth due to increasing atmospheric CO₂ concentration is hypothesized to further increase this terrestrial C sink (Prentice et al., 2001). Free air CO₂ enrichment experiments in aggrading forests and plantations have demonstrated significant increases in net primary production (NPP) and C storage in forest vegetation (Calfapietra et al., 2003; DeLucia et al., 1999; Gielen et al., 2005; Hamilton et al., 2002; Liberloo et al., 2006; Norby et al., 2005; Norby et al., 2002). The extra C uptake may, next to forest vegetation, also be stored in forest floor litter and in forest soil. The fate of this additional C allocated belowground remains unclear (Jastrow et al., 2005; Lichter et al., 2005; Norby et al., 2002; Schlesinger and Lichter, 2001). Enhanced carbon transfer to the root system may result mainly in enhanced root respiration or, otherwise, in an increase of root dry matter, mycorrhizal activity and subsequent transfer of carbon to soil C pools.

The stability of soil organic matter is controlled by the chemical structure of the organic matter and the existence of protection offered by the soil matrix and minerals (Baldock and Skjemstad, 2000; Davidson and Janssens, 2006;

Correspondence to: M. R. Hoosbeek
(marcel.hoosbeek@wur.nl)

Elliott, 1986; Jastrow, 1996; Krull et al., 2003; Six et al., 2002; Van Veen and Kuikman, 1990). The additional C input into the soil may affect population size and activity of soil fauna and flora, and may therefore also affect the formation of soil aggregates (Oades, 1993). It has been established that the inclusion of organic matter within aggregates reduces its decomposition rate (Krull et al., 2003; Oades, 1984; Six et al., 2002, 2000; Tisdall and Oades, 1982). Oades (1984, 1993) suggested a model of aggregate formation in which micro-aggregates ($\sim 100 \mu\text{m}$ in diameter) are formed within macro-aggregates ($> 250 \mu\text{m}$ in diameter). This model of the *cycle of aggregate formation* has been extended and applied by Jastrow (1996), Puget (1995) and Six et al. (2002, 2001, 1999, 1998). Fresh plant remains entering the soil become sites for microbial activity and nucleation centers for aggregation. The enhanced microbial activity induces the binding of organic matter and soil particles into macro-aggregates. As the enclosed organic matter is decomposed, microbial and decomposition products become associated with mineral particles (Chenu and Stotzky, 2002). This association results in the formation of micro-aggregates. Eventually, the binding agents in macro-aggregates degrade, resulting in a breakdown of macro-aggregates and the release microbially processed organic matter and micro-aggregates. Six et al. (1999) hypothesized that these released micro-aggregates are a mixture of old micro-aggregates, which had been previously formed and incorporated during macro-aggregate formation, and newly formed micro-aggregates.

At two sites in Ohio and Ontario Six et al. (2002) found that afforestation of cultivated land resulted in increased aggregation and a greater C stock in the A horizons. The micro-aggregates and their capacity to protect C in the longer term were found to be crucial for C sequestration in both forested systems. Twenty years after afforestation of former arable land in northeastern Italy, Del Galdo et al. (2003) observed an increase of soil C of respectively 23 and 6% at 0–10 and 10–30 cm soil depth. Moreover, afforestation resulted in stabilization of soil C in micro-aggregates.

After six years of CO₂ enrichment at the Duke Forest FACE experiment, Lichter et al. (2005) did not detect a significant FACE effect on soil C content. However, the C content of the mineral top soil (0–15 cm) averaged over the FACE and control rings significantly increased during the experiment. Physical fractionation suggested that this increase occurred entirely within the free light fraction in which organic C is not protected against decomposition. Fractions in which soil C is protected to some degree, i.e. coarse and fine intra-aggregate particulate organic matter (iPOM) and mineral associated organic matter (micro-aggregates) were not affected by FACE. At the Oak Ridge deciduous forest FACE experiment, organic C in the surface 5 cm of the soil increased linearly during 5 years of CO₂ enrichment, while C in the ambient plots remained relatively constant (Jastrow et al., 2005).

After 3 years of FACE treatment at the POPFACE poplar plantation, significantly more new C was incorporated into the mineral soil (Hoosbeek et al., 2004). We also observed a significantly larger increase in total soil C after 5 years of CO₂ enrichment (Hoosbeek et al., 2006). Chemical fractionation revealed a FACE induced increase of the labile soil C fraction. We hypothesize that this extra labile soil C will increase the formation of macro-aggregates and subsequently will increase the formation of micro-aggregates. This FACE enhanced physical protection of soil organic matter may increase long-term C sequestration in forest soils.

2 Methods

The POPFACE experiment was established early 1999 on former agricultural fields near Viterbo (42°37'04" N, 11°80'87" E, alt 150 m), Italy. The plantation and adjacent fields had been under forest until about 1950. Since then a variety of agricultural crops has been grown on these former forest soils until the inception of the POPFACE plantation. The annual precipitation is on average 700 mm with dry summers (Xeric moisture regime). During November of 1998 an initial soil survey took place. The loamy soils classified as Pachic Xerumbrepts and were described in detail by Hoosbeek et al. (2004).

Nine ha were planted with *Populus x euramericana* hardwood cuttings at a density of 0.5 trees per m². Within this plantation three FACE and three control plots (30×30 m) were randomly assigned under the condition of a minimum distance between plots of 120 m to avoid CO₂ cross-contamination. These six plots were planted at a density of 1 tree per m² using three different genotypes. 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. However, because of the high inorganic N content of the soil, no fertilization treatment was applied during the first 3-year rotation of the experiment. Each half plot was divided into three sectors, where each sector was planted at a density of 1 tree per m² using three different genotypes: *P. x euramericana* Dode (Guinier) (= *P. deltoides* Bart. ex Marsh. x *P. nigra* L.) genotype I-214, a genotype of *P. nigra* L. (Jean Pourtet) and a local selection of *P. alba* L. (genotype 2AS11). Carbon enrichment was achieved by injection of pure CO₂ through laser-drilled holes in tubing mounted on six masts (Miglietta et al., 2001). The FACE rings (octagons) within the FACE plots had a diameter of about 22 m. The elevated CO₂ concentrations, measured at 1-min intervals, were within 20% deviation from the pre-set target concentration ($560 \mu\text{mol mol}^{-1}$) for 91% of the time to 72.2% of the time, respectively, at the beginning and at the end of each rotation cycle of the plantation. The plantation was drip irrigated at a rate of 6 to 10 mm per day during the growing seasons.

The trees were coppiced after the first three growing seasons (1999–2001). The experiment continued with a second rotation under the name EuroFACE (2002–2004). A fertilization treatment was added to one half of each experimental plot because soil analyses showed the occurrence of limiting conditions of nitrogen availability in the soil (Scarascia-Mugnozza et al., 2006). The total amount of nitrogen supplied was 212 kg ha⁻¹ y⁻¹ in 2002 and 290 kg ha⁻¹ y⁻¹ during 2003 and 2004.

Soil samples were collected from each sector within 2 control (rings 2 and 3) and 2 FACE plots (rings 1 and 4) in October of 2003. Bulk density samples, one per sector, were taken at 0–10 cm below the surface of the mineral soil with the help of a bulk density sampler that holds 300 cm³ metal rings. The samples were dried at 105°C for 3 days. Bulk densities were calculated based on dry weight and ring volume. Next, the soil samples were crushed by hand and live roots were removed. No carbonates were present in the soil. Carbon and nitrogen were determined by flash combustion in an elemental analyzer (EA 1108) (Van Lagen, 1996). Total soil organic C and N content are expressed as weight percentage (g C or N per gram soil × 100%).

2.1 Whole sample fractionation

For fractionation, one bulk sample per sector was collected with a small spade from the upper 10 cm of the mineral soil and air dried at room temperature. Before drying, large aggregates (>1 cm) were broken up along natural planes of weakness. The wet sieving procedure was described by Kemper and Roseau (1986) and Pulleman et al. (2003). Materials used included a wet sieving apparatus, 20 l buckets (used as wet sieving basins) and four 20 cm diameter sieves (2000, 1000, 250 and 53 μm mesh). The buckets were filled with demineralized water; the sieves were stacked, submerging one sieve at a time to prevent air bubbles from getting trapped under a sieve. The top sieve (2000 μm) was placed on top of the stack without touching the water at first. Dried soil material was placed on the top sieve, after which the stack of sieves was lowered until the material on the top sieve was just covered by water. The samples were left to slake for 5 min, followed by 2 min of wet sieving. The wet sieving apparatus gently lowers and lifts the sieves at a speed of about 30 repetitions per minute, over a distance of 3 cm. After sieving, the sieves were lifted out of the water and the material that remained on the sieves was washed into beakers assigned to the specific fractions. The isolated fractions were dried at 40°C. Four fractions based on the following size classes were distinguished: 53–250 (micro-aggregates), 250–1000 (small macro-aggregates), 1000–2000 (medium macro-aggregates), and >2000 μm (large macro-aggregates).

Six et al. (1998) used three sieves: 2000, 250 and 53 μm, while we used an additional 1000 μm sieve. The reason for this was the relatively large 250–2000 μm fraction of the samples we used, which tended to block the 250 μm sieve.

We also used 75 g of sample per stack of sieves instead of 100 g in order to prevent blockage.

In general, differences in texture between field plots and aggregate fractions, and the fact that there is hardly any binding between sand particles and organic matter, makes sand fraction correction a necessity when comparing aggregate fractions and their C and N contents (Elliott et al., 1991; Six et al., 1998). Per obtained fraction, 2 g of soil material was used. The aggregates were destroyed by removing organic matter with peroxide and dispersing clay with a dispersing solution of sodium carbonate and sodium polyphosphate. Dispersion was completed by ultrasonic treatment (Van Doesburg, 1996). Next, the destroyed size fraction was washed over the original sieve. The material left on the sieve was taken as the sand fraction. Aggregate weight fractions were calculated as:

Aggregate weight fraction_(1..4) =

$$\frac{\text{total weight fraction}_{(1..4)} - \text{weight sand fraction}_{(1..4)}}{\text{total sample weight}}$$

C and N contents could only be determined for uncorrected fractions (because the sand correction includes the loss of organic material). However, we assumed that the sand fractions of the aggregate fractions and total sample did not contain C or N (just inert minerals). The aggregate C fractions were calculated as:

$$\text{Aggregate C fraction}_{(1..4)} = \frac{\text{g C fraction}_{(1..4)}}{\text{g C total sample}}$$

The aggregate N fractions were calculated in a similar fashion.

2.2 Intra-macro-aggregate (iM) fractions

Next, the small and medium sized macro-aggregate fractions were combined into one 250–2000 μm macro-aggregate fraction. Breaking up this macro-aggregate fraction will, according to the aggregate formation model, result in the release of clay and silt (<53 μm), micro-aggregates (53–250 μm), and coarse POM plus sand (>250 μm). These intra-macro-aggregate fractions will be indicated by “iM”. A “micro-aggregate isolator”, as described by Six et al. (2002), was used to break up the macro-aggregates while minimizing the break down of the released iM-micro-aggregates. Ten grams of macro-aggregates were immersed in deionized water on top of a 250 μm mesh screen and shaken with 50 glass beads (4 mm diameter). A continuous water flow through the device flushed all released iM-micro-aggregates immediately onto a 53 μm sieve, thus avoiding further disruption. After complete breakup of the macro-aggregates, coarse iM-POM and sand remained on the 250 μm mesh screen. The iM-micro-aggregates and the iM-clay and silt fraction were separated by the 53 μm sieve. The iM-micro-aggregate weight fractions were calculated as:

Table 1. Carbon and nitrogen weight percentages of the mineral top soil in the sampled rings (1–4), October 2003.

Treatment		C %			N %		Bulk density (g soil cm ⁻³)	
		n	mean	s.e.	mean	s.e.	mean	s.e.
CO ₂	Ambient	2	1.18	0.04	0.11	0.00	1.26	0.02
	FACE	2	1.20	0.03	0.13	0.01	1.17	0.04
N	Ambient	4	1.20	0.04	0.12	0.01	1.21	0.03
	Fertilized	4	1.18	0.03	0.12	0.00	1.23	0.04
Species	<i>alba</i>	8	1.17	0.04	0.12	0.01	1.20	0.05
	<i>nigra</i>	8	1.17	0.04	0.12	0.01	1.22	0.05
	<i>euramericana</i>	8	1.23	0.05	0.13	0.01	1.23	0.04

Table 2. Effect of FACE, N-fertilization and species on sand free aggregate weight fractions.

Treatment		Aggregate weight fractions								
		n	53–250 μm		250–1000 μm		1000–2000 μm		>2000 μm	
			mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
CO ₂	Ambient	2	0.09	0.01	0.33	0.02	0.15	0.01	0.22	0.02
	FACE	2	0.09	0.01	0.33	0.02	0.15	0.01	0.24	0.03
N	Ambient	4	0.09	0.01	0.31	0.02	0.15	0.01	0.24	0.02
	Fertilized	4	0.09	0.01	0.35	0.02	0.15	0.01	0.22	0.03
Species	Alba	8	0.08	0.01	0.28	0.03	0.17	0.01	0.27	0.03
	Nigra	8	0.08	0.01	0.31	0.01	0.15	0.01	0.26	0.02
	Euramericana	8	0.10 a	0.01	0.40 b	0.02	0.13 c	0.01	0.16 d	0.03

- a. Significant species effect (P=0.002) on 53–250 μm aggregate weight fraction.
 b. Significant species effect (P=0.007) on 250–1000 μm aggregate weight fraction.
 c. Significant species effect (P=0.005) on 1000–2000 μm aggregate weight fraction.
 d. Significant species effect (P=0.018) on >2000 μm aggregate weight fraction.

iM micro aggregate weight fraction =

$$\frac{\text{g iM aggregate fraction}_{53-250 \mu\text{m}} - \text{g iM sand fraction}_{53-250 \mu\text{m}}}{\text{g macro aggregate fraction}_{250-2000 \mu\text{m}}}$$

The iM-micro-aggregate C fractions were calculated as:

iM micro aggregate C fraction =

$$\frac{\text{g C iM aggregate fraction}_{53-250 \mu\text{m}}}{\text{g C macro aggregate fraction}_{250-2000 \mu\text{m}}}$$

The iM-micro-aggregate N fractions were calculated similarly.

2.3 Statistical analysis

The EuroFACE field experiment was set up as a split-split-plot design, i.e. the CO₂ treatments are the whole-plots (ambient and FACE rings), which have been split into two N-fertilization treatments, and these sub-plots were then further split into three sub-sub-plots with different poplar genotypes.

At the inception of the experiment, the whole-plots were assigned randomly within the poplar plantation. In order to account for this we included a random “location” factor in our statistical model. Location was defined as the whole-plot (ring) number (RingNr) within the CO₂ treatment. We used the general linear model (SPSS 12.0.1) with the following design: CO₂trmt (fixed); Ntrmt (fixed); Species (fixed); RingNr(CO₂trmt) (random); CO₂trmt × Ntrmt; Ntrmt × RingNr(CO₂trmt); CO₂trmt × Species; Ntrmt × Species; CO₂trmt × Ntrmt × Species, where RingNr(CO₂trmt) indicates the nested structure of the data. For practical reasons, i.e. limiting the amount of soil fractionation work, we used samples from 4 whole-plots (ambient rings 2 and 3; FACE rings 1 and 4). The number of replicates per treatment are therefore: CO₂ treatment *n*=4 (2 ambient + 2 FACE); N treatment *n*=8 (4 unfertilized + 4 N-fertilized); Species *n*=24 (8 *P. alba* + 8 *P. nigra* + 8 *P. euramericana*). Main or interaction effects were considered to be significant when the P-value of the F-test was <0.05.

Table 3. Effect of FACE, N-fertilization and species on aggregate C fractions.

Treatment		Aggregate C fractions								
		N	53–250 μm		250–1000 μm		1000–2000 μm		>2000 μm	
			mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
CO ₂	Ambient	2	0.13	0.01	0.46	0.03	0.18	0.01	0.26	0.03
	FACE	2	0.11	0.01	0.42	0.03	0.19	0.01	0.29	0.04
N	Ambient	4	0.12	0.01	0.41	0.03	0.18	0.01	0.29	0.03
	Fertilized	4	0.12	0.01	0.47	0.03	0.19	0.01	0.26	0.03
Species	Alba	8	0.11	0.01	0.39	0.04	0.20	0.02	0.31	0.04
	Nigra	8	0.12	0.01	0.42	0.03	0.19	0.01	0.32	0.03
	Euramericana	8	0.14 a	0.01	0.51 b	0.03	0.16 c	0.01	0.19 d	0.03

- a. Significant species effect ($P=0.002$) on 53–250 μm aggregate carbon fraction.
 b. Significant species effect ($P=0.029$) on 250–1000 μm aggregate carbon fraction.
 c. Possible species effect ($P=0.090$) on 1000–2000 μm aggregate carbon fraction.
 d. Significant species effect ($P=0.026$) on >2000 μm aggregate carbon fraction.

Table 4. Effect of FACE, N-fertilization and species on aggregate N fractions.

Treatment		Aggregate C fractions								
		N	53–250 μm		250–1000 μm		1000–2000 μm		>2000 μm	
			mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
CO ₂	Ambient	2	0.09	0.01	0.32	0.02	0.15	0.01	0.23	0.04
	FACE	2	0.09	0.01	0.30	0.02	0.14	0.01	0.22	0.03
N	Ambient	4	0.09	0.01	0.32	0.02	0.14	0.01	0.24	0.03
	Fertilized	4	0.08	0.01	0.31	0.02	0.15	0.01	0.22	0.03
Species	Alba	8	0.08	0.01	0.30	0.03	0.17	0.01	0.27	0.04
	Nigra	8	0.10	0.01	0.33	0.03	0.17	0.01	0.29	0.03
	Euramericana	8	0.08	0.01	0.31	0.03	0.10 a	0.01	0.12 b	0.01

- a. Significant species effect ($P=0.001$) on 1000–2000 μm aggregate nitrogen fraction.
 b. Significant species effect ($P=0.007$) on >2000 μm aggregate nitrogen fraction.

3 Results

At the end of the fifth growing season, average soil C and N percentages of the top 10 cm of the mineral soil in the sampled rings (1–4) were 1.19 and 0.12% respectively (Table 1). The average bulk density was 1.22 g soil cm⁻³.

3.1 Whole sample fractionation

The small macro-aggregate (250–1000 μm) weight fraction was the largest fraction making up one-third of the total sample weight (Table 2). The second largest weight fraction was the large macro-aggregate (>2000 μm) fraction. FACE and N fertilization treatments had no effect on aggregate weight fractions. However, differences in species did change the weight distribution among the aggregate fractions significantly. *Populus euramericana*, as compared to *P. alba* and *nigra*, increased the free micro-aggregate (53–250 μm) and

small macro-aggregate fractions and decreased the medium and large macro-aggregate fraction.

Based on C content, the small macro-aggregate aggregate fraction was again the largest holding on average 44% of the carbon (Table 3). FACE and N fertilization treatments had no effect on the C distribution. However, *P. euramericana* increased the free micro- and small macro-aggregate C fraction while it decreased the medium and large macro-aggregate C fraction.

Most N was contained in the small macro-aggregate fraction, making up almost one-third of the total (Table 4). Again, only a species effect was observed. *P. euramericana* decreased the medium (1000–2000 μm) and large macro-aggregate N fractions.

Table 5. Isolated iM-micro-aggregate weight, C and N fractions.

Treatment		iM-micro-aggregate						
		N	weight fraction		C fraction		N fraction	
			mean	s.e.	mean	s.e.	mean	s.e.
CO ₂	Ambient	2	0.53	0.01	0.63	0.01	0.73	0.06
	FACE	2	0.60	0.03	0.71	0.02	0.75	0.03
N	Ambient	4	0.57	0.03	0.66	0.02	0.70	0.05
	Fertilized	4	0.57	0.02	0.67	0.02	0.78	0.04
Species	Alba	8	0.56	0.03	0.68	0.03	0.68	0.05
	Nigra	8	0.56	0.03	0.66	0.02	0.66	0.04
	Euramericana	8	0.58	0.04	0.67	0.02	0.87 ^a	0.05

a. Significant species effect ($P=0.003$) on iM-micro-aggregate N fraction.

3.2 Intra-macro-aggregate (iM) fractions

The combined small and medium macro-aggregates consisted of, on average by weight percentage, 57% iM-micro-aggregates, 24% iM-coarse POM and sand, and 15% iM-clay and silt particles. FACE and N fertilization treatments and poplar species had no significant effect on the iM-aggregate weight fractions (Table 5; iM-clay and silt and iM-coarse POM and sand data not shown). FACE did however, increase the iM-micro-aggregate weight fraction from 0.53 (ambient) to 0.60 (FACE).

Similarly, FACE and N fertilization treatments and poplar species had no significant effect on the iM-aggregate C fractions, although FACE increased the iM-micro-aggregate C fraction. The iM-N fractions were not affected by either FACE or N treatment. However, the iM-micro-aggregate N fraction increased under *Populus euramericana*.

4 Discussion

In preparation of the POPFACE experiment the experimental field was ploughed during the Fall of 1998 (Hoosbeek et al., 2004). At that time the structure of the loamy A horizon was characterized by coarse prismatic peds (FAO, 1990). Five years after establishment of the plantation, three litter layers (L, F and H) had formed on top of the mineral soil. Underneath, the structure of the A horizon had changed into fine and medium sized blocky and granular aggregates. In the F, H and A horizons many arthropods and a few earthworms were observed. Afforestation obviously changed the structure of the top soil within 5 years.

We hypothesized that FACE treatment, through an increase of net primary production and increased C input into the soil, would increase the formation of macro-aggregates and subsequently the formation of micro-aggregates. We also expected N-fertilization to have a positive effect on aggregate formation because of increased N availability to plants and soil flora and fauna.

4.1 Whole sample fractionation

The results of whole sample fractionation showed no FACE or N fertilization effect on aggregate formation. Instead, a species effect was observed. Fewer medium and large sized and more small sized macro-aggregates were present under *P. euramericana*. Moreover, *P. euramericana* increased the formation of free micro-aggregates. According to the model of aggregate formation, in which micro-aggregates are formed within macro-aggregates, this increase of free micro-aggregates under *P. euramericana* may be explained in two ways: 1) More medium and large sized macro-aggregates broke up prior to sampling which increased the number of released micro-aggregates. 2) More small sized macro-aggregates are being formed and subsequently more micro-aggregates are being released. Explanation 1 assumes increased disruption or degradation of large macro-aggregates under *P. euramericana*. Also, it assumes that the medium and large macro-aggregate fractions were not replenished by newly formed medium and large macro-aggregates. Explanation 2 assumes an increase in the speed of the cycle of aggregate formation, resulting in an increase of macro-aggregate formation and subsequently an increase of micro-aggregate formation. Explanation 1 seems unlikely because there is no good reason for increased disruption of medium and large macro-aggregates and the subsequent lack of formation of new medium and large sized macro-aggregates under *P. euramericana*. Explanation 2 is supported by estimates of fresh plant remains entering the soil. Lukac et al. (2003) calculated the amount of C transferred into the soil via fine roots during the first rotation (POPFACE) as a result of fine root production and turnover. They estimated the C input for respectively *P. alba*, *nigra* and *euramericana* under ambient CO₂ to be: 124, 128 and 170 (g/m²); and under FACE respectively: 189, 232 and 309 (g/m²). The actual amount of C entering the soil was thought to be higher because root respiration and exudation was not accounted for. However, on average, under ambient CO₂ and FACE, 42% more C entered

the soil via fine roots under *P. euramericana* as compared to under *alba* and *nigra*. Hoosbeek et al. (2004) estimated the input of C into the soil with the C3/C4 stable isotope method during the second and third year. Again, most C entered the soil under *P. euramericana*, i.e. on average 16 and 15% more new C in respectively 2000 and 2001 as compared to *P. alba* and *nigra*. The observed larger C inputs and increase of small macro-aggregate and free micro-aggregate fractions under *P. euramericana* are in agreement with the *cycle of aggregate formation* model. The larger input of fresh litter under *P. euramericana* increased the number of sites for microbial activity and the number of nucleation centers for aggregation. Enhanced microbial activity induced an increase of binding of organic matter and soil particles into macro-aggregates. More enclosed organic matter was decomposed and was subsequently associated with mineral particles. Increased association resulted in increased formation of micro-aggregates and long term stabilization of soil organic matter.

As with the weight fractions, the free micro- and small macro-aggregate C fractions increased under *P. euramericana*, while the medium and large macro-aggregate C fractions decreased. The medium and large macro-aggregate N fractions also decreased under *P. euramericana*. A species effect on the micro- and small macro-aggregate N fractions was probably obscured by the relatively high C/N ratio of the fresh plant material as compared to the lower C/N ratio of the older organic matter included in the macro-aggregates.

The absence of a N-fertilization effect suggests that N availability is not a limiting factor to the soil flora and fauna involved in processes related to aggregate formation.

4.2 Intra-macro-aggregate (iM) fractions

The isolated iM-micro-aggregate weight and C fractions were larger under FACE than under ambient CO₂, although not significantly (Table 5). This lack of significance may in part be due to the limited statistical power of the split-split-plot design with respect to the CO₂ treatment. If we had assumed the six soil samples per plot (CO₂ treatment ring) to be independent, then the iM-micro-aggregate C fraction would have been significantly ($P=0.003$) larger under FACE. This result may seem to contradict the fact that the macro-aggregate fractions, that harbored these iM-micro-aggregate fractions, were not affected by FACE. However, the fractions remaining on the sieves after wet sieving represent aggregate fractions with a certain minimum aggregate stability. Similar weight fractions may still include aggregates with different stabilities above a certain minimum. Although the macro-aggregate fractions were not affected by FACE, the average stability of the macro-aggregate fractions formed under FACE may have been higher. A higher stability causes a slower macro-aggregate turnover, which enhances the formation of iM-micro-aggregates inside (Six et al., 2000). So, after five years of treatment, FACE may have increased the formation of the iM-micro-aggregate C fraction probably due

to increased stability of macro-aggregates. Eventually, these iM-micro-aggregates may be released from the “nursery” and may increase the “free” micro-aggregate C fraction as fraction of the whole soil. Through this mechanism, FACE may increase the stabilization and protection of soil C in iM-micro-aggregates, and may eventually, as we expect, also increase the stabilization and protection of soil C in “released” micro-aggregates. However, as mentioned, this trend can not be confirmed with the statistical model that we used based on the split-split-plot design.

Another factor affecting the results of the iM-macro-aggregate fractions may have been the inclusion of small amounts of fine (53–250 micron) inter-micro-aggregate particulate organic matter (POM) released through the dispersion of macro-aggregates or unstable micro-aggregates. We assumed this POM material to be negligible as compared to the isolated iM-micro-aggregate fraction, especially on weight basis. But, the C and N contents may have been affected to some extent, implying that not all obtained C and N was truly protected.

4.3 Forest FACE experiments

Until recently, increased atmospheric CO₂ concentration was reported to have no significant effect on soil C sequestration at forest FACE experiments (Houghton, 2003; Lichter et al., 2005; Norby et al., 2002; Schlesinger and Lichter, 2001). After six years of CO₂ enrichment at the Duke Forest FACE experiment, Lichter et al. (2005) did not detect a significant FACE effects on soil C content. However, the C content of the mineral top soil (0–15 cm) averaged over the FACE and control rings significantly increased during the experiment due to regrowth. Physical fractionation suggested that this increase occurred entirely within the free light fraction in which SOM is not protected against decomposition. The iPOM and iM-micro-aggregate C fractions were not affected by FACE. Lichter et al. (2005) concluded that forest soils are unlikely to sequester significant additional quantities of atmospheric C associated with CO₂ fertilization because of the low rates of C input to refractory and protected SOM pools.

Recently, Jastrow et al. (2005) raised the question whether the lack of a FACE effect on soil C content is a general response or a function of (1) the low statistical power of most experiments, and/or (2) the magnitude of CO₂-stimulated C inputs relative to the duration of the experiments. At the Oak Ridge deciduous forest FACE experiment, organic C in the surface 5 cm of the soil increased linearly during 5 years of CO₂ enrichment, while C in the ambient plots remained relatively constant (Jastrow et al., 2005). A significant FACE effect on soil C was observed for the top 5 cm. Sampling of a thicker soil increment, e.g. 0–15 cm, would have “diluted” the increase of C which would have resulted in a non-significant effect. A meta-analysis of 35 independent experimental observations from a wide range of ecosystems showed that CO₂ enrichment increased soil C by 5.6%

(Jastrow et al., 2005). According to Jastrow et al. (2005), this result supports the generality of the observed increase of soil C under FACE at the Oak Ridge experiment.

At the EuroFACE site we also observed a significant larger increase in total soil C% after 5 years of CO₂ enrichment (Hoosbeek et al., 2006). Chemical fractionation revealed that this increase occurred within the labile soil C fraction. The question remained whether the observed larger increase of total soil C and the increase of labile soil C under FACE would eventually result in long-term C sequestration in stable organic matter fractions.

The results of physical fractionation presented in this study reveal that FACE did not affect the formation of aggregates. This lack of an effect may be due to the low statistical power of the split-split-plot design. We did, however, observe a trend of increased stabilization and protection of soil C in micro-aggregates formed within macro-aggregates under FACE. Moreover, the largest effect on aggregate formation was due to differences in species, i.e. poplar genotype. *P. euramericana* increased the formation of free micro-aggregates which means that more newly incorporated soil C was stabilized and protected. The choice of species in a plantation, or the effect of global change on species diversity, may therefore affect the stabilization and protection of C in soils.

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