Summary  A high-density plantation of three genotypes of *Populus* was exposed to an elevated concentration of carbon dioxide ([CO$_2$]; 550 µmol mol$^{-1}$) from planting through canopy closure using a free-air CO$_2$ enrichment (FACE) technique. The FACE treatment stimulated gross primary productivity by 22 and 11% in the second and third years, respectively. Partitioning of extra carbon (C) among C pools of different turnover rates is of critical interest; thus, we calculated net ecosystem productivity (NEP) to determine whether elevated atmospheric [CO$_2$] will enhance net plantation C storage capacity. Free-air CO$_2$ enrichment increased net primary productivity (NPP) of all genotypes by 21% in the second year and by 26% in the third year, mainly because of an increase in the size of C pools with relatively slow turnover rates (i.e., wood). In all genotypes in the FACE treatment, more new soil C was added to the total soil C pool compared with the control treatment. However, more old soil C loss was observed in the FACE treatment compared with the control treatment, possibly due to a priming effect from newly incorporated root litter. FACE did not significantly increase NEP, probably as a result of this priming effect.

Keywords: global change, NEP, NPP, Populus.

Introduction  The ongoing anthropogenic increase in atmospheric carbon dioxide concentration ([CO$_2$]) is a primary factor in climate change (Houghton et al. 2001). Mitigation of the increase in atmospheric [CO$_2$] is an important goal for humanity. Terrestrial ecosystems, and in particular forest ecosystems, are the focus of intensive research on the effects of elevated [CO$_2$] because of their importance in the global carbon (C) cycle, (DeLucia et al. 1999, Hendrey et al. 1999, Isebrands et al. 2001, Norby et al. 2001). Forest plantation fossil C storage capacity is of particular interest as a [CO$_2$] reduction strategy, for the fulfillment of Kyoto Protocol requirements (Article 3.3, UNFCC 1997). Among various agricultural land-use and management strategies, bioenergy crops have the largest potential for C storage (Smith et al. 2000). Fast-growing tree species such as poplar are important components of temperate and boreal ecosystems and are especially well suited for plantation culture. Short-rotation culture (SRC) of poplar is increasingly used as a source of renewable energy; other applications include removal of excess fertilizers and phytoremediation (Isebrands and Karnosky 2001).

Experiments using the free-air CO$_2$ enrichment (FACE) technique provide a powerful tool for the study of whole-eco-
system C budgets. To date, only two FACE-exposed forest ecosystem C budgets have been published; however, both studies used stepwise [CO₂] increases in established stands. The C budget may be fundamentally different in forest ecosystems established and grown in elevated [CO₂]. Net primary productivity (NPP) of an intact Pinus taeda L. forest increased by 25% after 2 years of FACE, a stimulation that was sustained over 4 years (DeLucia et al. 1999, Hamilton et al. 2002). This increase was caused by increased tree growth rates (Hamilton et al. 2002). Similarly, FACE increased NPP of a closed-canopy deciduous forest (Nobry et al. 2002). However, although most of the additional net C uptake in the first year was allocated to woody biomass, subsequent years saw a shift from woody biomass to fine-root production (Nobry et al. 2002). Because wood has a much slower turnover rate than fine roots (Gaudinski et al. 2001, Nobry et al. 2002), this finding has important implications for net forest C storage capacity. Nevertheless, increased fine-root productivity may also serve an important role in the long-term accumulation of C in terrestrial ecosystems by adding C to the soil, thereby ultimately increasing C stores in long-lived soil organic matter (SOM) pools (Lukac et al. 2003).

Much of the current knowledge about forest ecosystem C source and sink strength is based on estimates of net ecosystem C fluxes, but our understanding of forest C budgets at the process level is poor. In both chamber as well as FACE experiments, attempts to quantify soil C stores have found only small changes in response to increased [CO₂] (Leavitt et al. 1994, Hungate et al. 1996, Schlesinger and Lichter 2001). However, because of the magnitude and high spatial variation of soil C stores, quantifying changes is difficult. Many CO₂ enrichment systems use petrochemically derived CO₂, which is depleted in δ¹³C, thus functioning as a tracer of C fluxes between vegetation and soils. Several studies have made use of this tracer to quantify C input from the vegetation to the soil (Leavitt et al. 1994, Hungate et al. 1996, Schlesinger and Lichter 2001). However, because of the magnitude and high spatial variation of soil C stores, quantifying changes is difficult. Many CO₂ enrichment systems use petrochemically derived CO₂, which is depleted in δ¹³C, thus functioning as a tracer of C fluxes between vegetation and soils. Several studies have made use of this tracer to quantify C input from the vegetation to the soil (Leavitt et al. 1994, Hungate et al. 1996), but it was impossible to assess control treatments in these studies. To overcome this limitation, we used the C3/C4 stable isotope method (Balesdent et al. 1988, Ineson et al. 1996). The CO₂ gas used for fumigation in our study had a δ¹³C signal of −6‰, close to the ambient value of −8‰. The method consists of growing C₃ plants (i.e., with a mean δ¹³C of −27‰) in C₄ soils (i.e., a soil developed under C₄ vegetation with a mean δ¹³C of −18‰), thus creating a tracer of plant-derived C input into the soil, independent of the elevated [CO₂] treatment.

In this paper, we report on the C budget of a FACE research site (POPFACE) where three Populus genotypes have been grown from planting through canopy closure in a high density plantation (Scarascia-Mugnozza et al. 2000, Miglietta et al. 2001). At the end of a 3-year growth period, the aboveground woody biomass was 15 to 27% higher in FACE plots than in control plots, depending on genotypes (Calfapietra et al. 2003). Belowground, FACE increased total root biomass by 47 to 76% (Lukac et al. 2003). The FACE treatment had a positive effect (50 to 97%) on the amount of plant-derived C incorporated into SOM during the third year (Hoosbeek et al. 2004).

Based on these findings, we hypothesized that the net C storage capacity of these plantations may increase in a future CO₂-enriched atmosphere. To test this hypothesis, we integrated relevant data from the FACE site to calculate NPP and net ecosystem productivity (NEP) for the second and third years.

Materials and methods

Description of the POPFACE site

The POPFACE experimental facility is located in central Italy (42°22’ N, 11°48’ E, 150 m a.s.l.) on a formerly agricultural, 9-ha field. The soil has a loam texture and a deep A horizon, and is classified as a Xeric Alfisol (Hoosbeek et al. 2004). After soil preparation, Populus × euramericana (Dode) Guinier (Clone I-214) hardwood cuttings were planted in late spring of 1999 at 2 × 1 m spacing. Six experimental plots were positioned within the plantation in a way that minimized enrichment pollution. Three control plots were left under natural conditions, whereas the remaining three plots had an elevated [CO₂] (550 µmol mol⁻¹) provided by the FACE technique. Measured FACE plot mean [CO₂] were 544 ± 48, 532 ± 83 and 554 ± 95 µmol mol⁻¹ in the first, second and third years, respectively. A detailed description of the FACE installation and of system performance is provided in Miglietta et al. (2001).

Each plot was 22 m in diameter and contained about 350 plants spaced at 1 × 1 m. Each plot was further divided into six sectors, of which two were planted with trees of a single Populus genotype. The genotypes used were: P. alba L. (Clone 2AS11), P. nigra L. (Clone Jean Pourtet) and P. × euramericana (P. deltoides Bart. ex Marsh. × P. nigra, Clone I-214). Further information on genotypic properties is detailed in Calfapietra et al. (2001). During the growing seasons, the plantation was drip-irrigated at a rate of 6 to 10 mm of water per day; weeds were removed manually or mechanically, and insecticides were applied as necessary.

Carbon pools and increments

In accordance with Hamilton et al. (2002), a C pool was defined as a C reservoir lasting 1 year or longer, and a C increment was defined as the annual change in the size of each pool. All data reported are for either the second (Year 2000) or third year (Year 2001) of the experiment.

Aboveground woody biomass

Following the method of Calfapietra et al. (2003), at the end of each year, the standing pool of aboveground woody biomass was calculated from genotype-specific allometric relationships established at the end of the second and third years by destructive harvests. Second-year allometric relationships were used to estimate biomass at the end of the first year. Biomass of the stump was determined from aboveground biomass using a mean genotype-specific ratio determined from harvested trees. Annual increments for both aboveground biomass and stump were determined from biomass at the end of each year minus biomass at the end of the previous year. Biomass was con-
Leaf litter production and decomposition

In the second and third years, litter production (g m⁻² of ground area) was monitored in control and FACE plots with 108 0.13-m² leaf litter traps, as described by Cotrufo et al. (2005). For the first year, leaf litter production was estimated from the total amount of leaf area produced (Gielen et al. 2001) and from mean values of specific leaf area. Leaf litter produced from July to December of the second and third year, was ground to a fine powder and analyzed for C concentration (Carlo Erba NA 1500, Carlo Erba Strumentazione, Milan, Italy) by sampling date and sector. Additionally, all litter produced from October to December of the second year was pooled by sector for chemical analyses and the decomposition study. Litter decomposition was monitored in the field with litter bags, and genotype and treatment decay rates were obtained. A detailed description of the experiment is in Cotrufo et al. (2005).

The annual increment in the standing leaf litter C pool was the cumulative value of the periodical C increments measured for that year, i.e., the weekly amount of litter fall multiplied by the corresponding value of C concentration. Annual C loss was calculated by multiplying the annual litter production by the yearly percentage mass loss. For the third year, C loss from the decomposition of the remaining first year litter was calculated by extrapolating the mass loss curves over a 2-year period. The leaf litter C pool was defined as the sum of C in the litter produced in that year and the C remaining on the forest floor after decomposition of the litter from previous years.

Root production and root turnover

Standing root biomass up to 40 cm in depth was sampled three to five times per season with a corer (inner diameter of 8 cm). Roots were divided into two diameter classes: fine (< 2 mm) and coarse (> 2 mm). Based on standard criteria, only negligible amounts of necromass were detected. A modification of the ingrowth-coring method, as described by Lukac and Godbold (2001), measured fine root production. Root turnover was determined according to Dahlman and Kučera (1965), as the ratio between annual production and maximum standing crop. These results are presented in detail in Lukac et al. (2003).

Carbon allocated to root system biomass was calculated based on coarse and fine root biomass data expressed per season, corrected for ash content and converted from dry biomass to C, based on actual C concentrations. Coarse root C increment was calculated as mean seasonal coarse root biomass minus the mean from the previous season, because no coarse root turnover was observed during the 3-year experimental period. To determine C allocation to fine roots, mean seasonal fine root biomass was multiplied by fine root turnover.

At the end of the third year, one tree in each sector was harvested and a subsample of the root system was analyzed for C by size class: fine (< 2 mm diameter), intermediate (2–5 mm) and coarse (> 5 mm).

Soil carbon

Data on soil C were derived from Hoosbeek et al. (2004). In November of all 3 years, soil samples were collected from each sector in all plots. Bulk density samples were taken by metal rings with a diameter and height of 10 cm, at 0–10, 10–20 and 20–30 cm soil depth. The samples were dried, crushed by hand and live roots removed. Carbon was measured with an elemental analyzer (Van Lagen 1996). Soil C pools were expressed per 0.1 m² using bulk densities, and summed over 0–30 cm soil depth to approximate soil C per m². Annual changes in soil C pools were calculated from the difference between soil C pools at the end of two consecutive years.

We used the C3/C4 stable isotope method outlined by Balesdent et al. (1988) to estimate annual influx of plant C (leaves and roots) to the soil. Root ingrowth cores (diameter of 4 cm, length of 40 cm, 2 mm mesh), filled with soil material with a C3-soil-like signature, were placed in the C3 soil of the plantation in March and collected in November of the second and third years. The fraction of C in the soil derived from leaves and roots during the incubation period (f) was calculated with a simple mixing model, as described by Balesdent et al. (1988). The amount of soil C newly accumulated (Cnew) during the incubation period was obtained by multiplying the C content of the incubated sample by f. From Cnew and from the change of the soil C pool between 2 years, the soil C loss per year was estimated. For further details see Hoosbeek et al. (2004).

Microbial biomass

Six soil cores (10 cm diameter, 20 cm depth) were taken from the 1–20 cm layer in each of the six plots, two per genotype, for a total of 36 soil cores on four sampling dates (March, June, August and October of the third year). Soil samples were immediately sieved (2 mm) and the water content adjusted to 60% of soil water holding capacity. Microbial biomass C was estimated by the fumigation extraction method (Vance et al. 1987). The results obtained for each sampling date were averaged and converted to g C m⁻² according to the soil bulk density of the layer 0–20 cm measured as described previously.

Gross primary productivity

Gross primary productivity (GPP) over the 3-year growth cycle was derived as described by Wittig et al. (2005). In brief, GPP was calculated with the equations of Long (1991) and the temperature corrections of Bernacchi et al. (2001, 2003b). The inputs were photon flux and temperature, measured at 30-min intervals from a nearby weather station, and leaf area index (Gielen et al. 2001, 2003), maximum rate of leaf carboxylation (Vmax) and electron transport (Jmax), determined previously throughout the growth cycle (Bernacchi et al. 2003a). From the meteorological conditions recorded at 30-min intervals and the biweekly leaf area index measurements, the microclimate of leaves within the plots was estimated with a radiation transfer and energy balance model. This information was in turn used as an input in the steady-state biochemical model of...
leaf photosynthesis (Farquhar et al. 1980) to compute leaf photosynthetic rate. The photosynthetic rates for different leaf classes were summed to obtain canopy photosynthesis, i.e., GPP. We estimated GPP for each species and each plot, and the means and standard errors for the three replicate plots are presented.

**Net primary productivity and net ecosystem productivity**

Annual NPP (g C m$^{-2}$ year$^{-1}$) was calculated as the summation of the increment of woody biomass (stem + stump + branches + coarse roots) + leaf litter production + fine root production. No data were available for branch litter and herbivory, but these are usually assumed to be negligible (DeLucia et al. 1999, Norby et al. 2002). Because the presence of insects and herbivores was insignificant (N. Anselmi and A. Olmi, Department of Plant Protection, University of Tuscia, Italy, personal communication), C losses due to herbivory were presumed to be minor. Branch litter was considered to be negligible during the first 3 years of the experiment.

Annual net C storage was calculated according to Curtis et al. (2002) from the increment of wood (stem, branches, stump) plus the increment of coarse roots plus changes in the soil C pool. Although no data for heterotrophic respiration were available to explicitly calculate NEP, annual net C storage is a valid approximation of NEP.

**Statistical analysis**

The main effects of CO$_2$ treatment, genotype and their interaction were determined by an analysis of variance (ANOVA). A randomized-complete-block design with treatment, genotype, and treatment $\times$ genotype interaction as fixed factors, and block as a random factor was applied. Plot was the unit of replication ($n = 3$). All statistical analyses were performed with the SAS statistical software package (SAS System 8.2, SAS Institute, Cary, NC) using the mixed procedure (Littell et al. 1996). In the case of a significant CO$_2$ treatment $\times$ genotype interaction, a posteriori comparison of means was performed with Bonferroni corrections for multiple comparisons. Differences between parameter means were considered significant when the $P$ value of the ANOVA $F$ test was $< 0.05$. Data were tested for normality using the Shapiro-Wilk statistic. If not otherwise stated, genotypes were significantly different and no CO$_2$ treatment $\times$ genotype interaction was found; we therefore focus on the effects of the FACE treatment.

**Results**

**Gross primary productivity**

Free-air CO$_2$ enrichment stimulated GPP in all genotypes by 22 and 11% in the second and third years, respectively ($P = 0.0002$ and 0.0019, respectively) (Table 1).

**Carbon pools**

Total plant C differed significantly between control and FACE treatments in both the second ($P = 0.0019$) and third years ($P = 0.0005$). Relative differences were in the range of 17–38% (second year) and 18–29% (third year) for the three genotypes (Figures 1A and 1B). With the exception of $P$. alba in the second year, relative treatment effects on plant C pools were a factor of 2.6–4 times larger for root than for stem and branch standing biomass (Figures 1A and 1B). The positive effects of FACE on fine root, coarse root, stump, stem and branch biomass were statistically significant (Calfapietra et al. 2003, Lukac et al. 2003). The leaf litter pool did not differ between treatments (second and third years) or between genotypes (second year) (Figures 1A and 1B). Stems and branches typically accounted for 61 to 74% of total plant C pools. In the third year, the ratio of stems + branches to total woody biomass was slightly lower in the FACE treatment than in the control treatment, i.e., 0.84 versus 0.88 for $P$. alba and $P$. nigra and 0.81 versus 0.84 for $P$. x euramericana ($P < 0.0001$; Figure 1B). Upon establishment of the plantation, the soil C pool reflected the site’s land-use history (Hoosbeek et al. 2004). There was considerable variation in the soil C pool among plots. At the end of the second year, in both control and FACE treatments, the soil C pool was nearly double that of the biomass C pool. After 3 years in FACE, the total plant biomass pools for $P$. alba, $P$. nigra and $P$. x euramericana were 3373, 4076 and 3403 g C m$^{-2}$, respectively, and were similar to, or exceeded, the soil C pools (down to 0.3 m; Figure 1B). At the end of the third year, the control treatment soil C pools still exceeded the biomass pools. Soil microbial biomass accounted for 1.7% (on average) of total soil C (Figure 1B). Although FACE did not affect microbial biomass, the microbial quotient, i.e., the fraction of microbial biomass over the total soil C, was 44% larger in the FACE plots than in the control plots ($P < 0.01$).

**Net primary productivity**

The NPP for all genotypes was increased by FACE by 21 (SE = 7%, $P = 0.0046$) and 26% (SE = 5%, $P = 0.0008$) in the second year.

**Table 1. Gross primary productivity (g C m$^{-2}$ year$^{-1}$) in high-density stands of three *Populus* genotypes in control and FACE treatments. Means (SE) of three plots for the second and the third years are presented and were obtained by modeling (Wittig et al. 2005). Carbon dioxide treatment effect was significant for both years.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control</th>
<th>FACE</th>
<th>Control</th>
<th>FACE</th>
<th>Control</th>
<th>FACE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Second year</td>
<td></td>
<td>Third year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P</em>. alba</td>
<td>1875 (35)</td>
<td>2297 (154)</td>
<td>2075 (66)</td>
<td>2476 (46)</td>
<td>1781 (36)</td>
<td>2204 (132)</td>
</tr>
<tr>
<td><em>P</em>. nigra</td>
<td>2585 (65)</td>
<td>2829 (128)</td>
<td>2773 (94)</td>
<td>2921 (36)</td>
<td>2150 (46)</td>
<td>2550 (39)</td>
</tr>
<tr>
<td><em>P</em>. x euramericana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Carbon pools in high-density stands of *P. alba*, *P. nigra* and *P. × euramericana* (*P. × eur*) in the second (A) and third (B) years under control and FACE treatments. All values are means (SE) in g C m$^{-2}$ ($n = 3$). Coarse roots are defined as > 2 mm, whereas fine roots are defined as < 2 mm. An explanatory legend is presented at the bottom of Figure 1A. Abbreviation: MB = microbial biomass.
and third years, respectively, compared with control conditions (Table 2). All components, except leaf litter, were significantly affected by FACE (Table 2). The coarse root increment in the second year was significantly larger in the FACE treatment only for *P. × euramericana* (*P* = 0.0030, *P* value of CO2 treatment × genotype = 0.0229). The effect of FACE on coarse root increment in the third year was marginally insignificant (*P* = 0.0509) because the coarse root increment of *P. × euramericana* was unaffected by FACE (*P* value of CO2 treatment × genotype = 0.1590). On a relative basis, FACE stimulated fine root production more than the other components of NPP, except for *P. alba*, in the third year. Fine root production of *P. alba* was not significantly affected by FACE in the third year. However, in the second year, stimulation of the above-ground woody component accounted for 65 (*P. alba*), 33 (*P. nigra*) and 54% (*P. × euramericana*) of the total FACE-induced increase in NPP. In the third year, these proportions increased to 67, 53, and 60% for *P. alba*, *P. nigra* and *P. × euramericana*, respectively.

Under control conditions, the NPP:GPP ratio in the second year was 0.60 (*P. alba*), 0.69 (*P. nigra*) and 0.64 (*P. × euramericana*), and in the third year, it was 0.58 (*P. alba*), 0.72 (*P. nigra*) and 0.71 (*P. × euramericana*). The FACE treatment had a positive effect (14%) on NPP:GPP in the third year (*P* = 0.0125).

**Litter and soil C fluxes**

Excess litter production in relation to decomposition resulted in litter accumulation. For *P. nigra* in the second year, the amount of C lost from the litter pool through decomposition was larger in the FACE treatment than in the control treatment (*P* = 0.0012, *P* value of CO2 treatment × genotype = 0.055; Table 2). Carbon loss from litter greatly increased in the third year, but did not differ between CO2 treatments or genotypes.

In the third year, more C<sub>new</sub> was accumulated in the mineral topsoil of FACE plots compared with control plots (*P* = 0.0062; Table 2), whereas there was no effect from FACE in the second year. Except for the third year of FACE treatment in *P. nigra* and *P. × euramericana*, the amount of mineral soil C increased each year (Table 2). FACE did not significantly affect changes in soil C or amounts of old soil C lost by respiration (Table 2). Although treatment means suggest much larger

### Table 2. Components of net primary productivity (NPP) and net yearly carbon (C) fluxes in high density stands of three *Populus* genotypes in control and FACE treatments. Means (SE) of three plots for the second and the third years are presented in g C m<sup>–2</sup> year<sup>–1</sup>. The *P* values of the CO2 treatment effect are indicated. Abbreviations: NEP = net ecosystem productivity (= increment of wood (stem, branches, stump) + increment of coarse roots + Δ soil C); litter C loss = C lost from the litter layer C pool due to decomposition of leaf litter; new soil C = new C input into soil C pool, from dead leaves, dead roots and mycorrhizal C; soil C loss = C loss from the soil C pool by respiration; and Δ soil C = new soil C – soil C loss.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO2 P value</th>
<th><em>P. alba</em></th>
<th><em>P. nigra</em></th>
<th><em>P. × euramericana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>FACE</td>
<td>Control</td>
</tr>
<tr>
<td>Above wood</td>
<td>0.0296</td>
<td>755 (52)</td>
<td>891 (102)</td>
<td>1035 (41)</td>
</tr>
<tr>
<td>Litter production</td>
<td>0.8283</td>
<td>208 (7)</td>
<td>216 (26)</td>
<td>224 (10)</td>
</tr>
<tr>
<td>Woody stump</td>
<td>0.0130</td>
<td>81 (6)</td>
<td>95 (11)</td>
<td>81 (3)</td>
</tr>
<tr>
<td>Coarse roots</td>
<td>0.0086</td>
<td>45 (13)</td>
<td>47 (8)</td>
<td>46 (7)</td>
</tr>
<tr>
<td>Fine roots</td>
<td>0.0001</td>
<td>43 (3)</td>
<td>92 (9)</td>
<td>52 (8)</td>
</tr>
<tr>
<td>Above/below NPP</td>
<td>0.0020</td>
<td>5.8 (0.6)</td>
<td>4.7 (0.1)</td>
<td>7.1 (0.7)</td>
</tr>
<tr>
<td>NPP</td>
<td>0.0046</td>
<td>1132 (57)</td>
<td>1341 (151)</td>
<td>1437 (31)</td>
</tr>
<tr>
<td>Litter C loss</td>
<td>0.006</td>
<td>3.15 (0.54)</td>
<td>4.95 (1.19)</td>
<td>5.47 (0.13)</td>
</tr>
<tr>
<td>New soil C</td>
<td>0.4949</td>
<td>469 (14)</td>
<td>594 (64)</td>
<td>572 (73)</td>
</tr>
<tr>
<td>Soil C loss</td>
<td>0.2259</td>
<td>352 (84)</td>
<td>495 (153)</td>
<td>396 (229)</td>
</tr>
<tr>
<td>Δ Soil C</td>
<td>0.3211</td>
<td>117 (77)</td>
<td>99 (88)</td>
<td>177 (157)</td>
</tr>
<tr>
<td>NEP</td>
<td>0.5103</td>
<td>998 (74)</td>
<td>1133 (168)</td>
<td>1338 (144)</td>
</tr>
</tbody>
</table>

### First year

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO2 P value</th>
<th><em>P. alba</em></th>
<th><em>P. nigra</em></th>
<th><em>P. × euramericana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>FACE</td>
<td>Control</td>
</tr>
<tr>
<td>Above wood</td>
<td>0.0120</td>
<td>1102 (11)</td>
<td>1462 (228)</td>
<td>1438 (43)</td>
</tr>
<tr>
<td>Litter production</td>
<td>0.2493</td>
<td>234 (12)</td>
<td>250 (14)</td>
<td>300 (24)</td>
</tr>
<tr>
<td>Woody stump</td>
<td>0.0001</td>
<td>40 (3)</td>
<td>122 (20)</td>
<td>43 (3)</td>
</tr>
<tr>
<td>Coarse roots</td>
<td>0.0509</td>
<td>22 (12)</td>
<td>80 (24)</td>
<td>110 (14)</td>
</tr>
<tr>
<td>Fine roots</td>
<td>0.0001</td>
<td>96 (5)</td>
<td>116 (9)</td>
<td>92 (4)</td>
</tr>
<tr>
<td>Above/below NPP</td>
<td>0.0033</td>
<td>8.6 (0.8)</td>
<td>5.4 (0.8)</td>
<td>7.2 (0.5)</td>
</tr>
<tr>
<td>NPP</td>
<td>0.0008</td>
<td>1493 (4)</td>
<td>2029 (232)</td>
<td>1983 (13)</td>
</tr>
<tr>
<td>Litter C loss</td>
<td>0.4681</td>
<td>36.40 (1.15)</td>
<td>37.30 (4.49)</td>
<td>35.88 (1.51)</td>
</tr>
<tr>
<td>New soil C</td>
<td>0.0062</td>
<td>294 (67)</td>
<td>525 (101)</td>
<td>319 (122)</td>
</tr>
<tr>
<td>Soil C loss</td>
<td>0.0741</td>
<td>113 (153)</td>
<td>365 (263)</td>
<td>234 (303)</td>
</tr>
<tr>
<td>Δ Soil C</td>
<td>0.4546</td>
<td>180 (95)</td>
<td>160 (211)</td>
<td>85 (184)</td>
</tr>
<tr>
<td>NEP</td>
<td>0.1733</td>
<td>1344 (92)</td>
<td>1824 (113)</td>
<td>1676 (211)</td>
</tr>
</tbody>
</table>
soil C losses in the FACE plots compared with the control plots, plot variation was too large to detect significant differences. New C input into the soil C pool, from dead leaves, dead roots and mycorrhizal C (New soil C), C loss from the soil C pool by respiration (soil C loss), and new soil C minus soil C loss (Δ soil C) did not differ among genotypes (Table 2).

*Net ecosystem productivity*

Partly because of considerable plot variation, there was no significant effect of FACE on NEP. In the second year, the mean stimulation of NEP by FACE for *P. alba* and *P. × eurameriana* was very small, and was nonexistent for *P. nigra* (Table 2). The effect of FACE on NEP was slightly larger in the third year, but not significant (Table 2). The effect of genotype on NEP was not significant.

**Discussion**

Averaged across genotypes, the irrigated high density plantation of poplar in a Mediterranean climate reached NPP values of 1233 and 1669 g C m\(^{-2}\) in the second and third years, respectively. Wood production at our site was within the range reported for other poplar plantations (Calfapietra et al. 2003), and NPP was similar to that of a sweetgum stand (Norby et al. 2002).

As a result of the sustained increase in leaf-level photosynthesis in response to FACE (Bernacchi et al. 2003a), GPP was 22 and 11% higher in the FACE plots than in the control plots in the second and third years, respectively (Wittig et al. 2005). As discussed by Wittig et al. (2005), the decline in the FACE-induced stimulation of GPP with time was a function of canopy closure without systematic down-regulation of photosynthetic capacity. In the context of net forest plantation C storage capacity, the partitioning of this extra C among pools of different turnover rates is of critical interest. Mean stimulation of NPP by FACE for the three poplar genotypes was comparable with the 21% stimulation of NPP in a sweetgum stand (Norby et al. 2002) and with the 27% increase in NPP in a *Pinus taeda* forest (Hamilton et al. 2002). In the first year, the FACE-induced increase in NPP in the sweetgum stand was attributed to an increase in the size of slow turnover (wood) C pools, but in the third year, it was attributed to an increase in the size of fast turnover C pools (leaves and fine roots) (Norby et al. 2002). At our site, in both the second and third years, more than 50% of the FACE-induced increase in NPP resulted from increased stem and branch increments. The contribution of fine root stimulation to the total FACE-induced increase in NPP was greater than the contribution of stems and branches only in *P. nigra* in the second year. Increased fine root production accounted for 4 to 30% of the FACE-induced increase in NPP in the other genotypes. Increased leaf litter accumulation accounted for less than 5% of the FACE-induced increase in NPP.

At the POPFACE site, litter was accumulating on the forest floor, as shown by Cotrufo et al. (2005). Leaf litter decay rates at the site, examined over an 8-month period, were low but not unusually so for the Mediterranean region (Moro and Domingo 2000, Cotrufo et al. 2005). However, afforestation of agricultural soil at the POPFACE site may have affected the capacity of the soil biological community to degrade a “new” substrate such as poplar litter, slowing down decomposition and resulting in fast litter accumulation, forming a distinct litter layer on top of the soil (Hoosbeek et al. 2004, Cotrufo et al. 2005). The microbial biomass accounted for 1.7% of the total soil C pool, which is low compared with the value of about 3% reported in similar studies (Rice et al. 1994, Hungate et al. 1997). This discrepancy may be associated with strong competition from poplar trees for nutrients, especially nitrogen, and the conversion of the site from agricultural use. Cotrufo et al. (2005) found slightly higher litter decay rates in the FACE plots, possibly because of increased rhizodeposition and soil biological activity (Hoosbeek et al. 2004). However, in elevated [CO\(_2\)], an increased amount of undecomposed litter is predicted to remain on the forest floor because it contains lower initial N concentrations (Cotrufo et al. 2005). It is presumed that this remaining litter slowly enters the SOM, and possibly promotes C storage; however, this process has not been verified in any FACE study. Although more litter accumulates in response to elevated [CO\(_2\)], no significant increase in long-lived SOM has been reported (Schlesinger and Lichter 2001). During our 3-year study, leaf litter contributed only a small amount to new soil C. Cumulative input of C from leaf litter, averaged across genotype and treatment, was 505 g C m\(^{-2}\). The mean input through leaf litter was larger than the mean input through fine root turnover by a factor of more than two. This ratio is similar to that calculated for Norway spruce (*Picea abies* L.) stands (Godbold et al. 2003) and many other forest ecosystems (Vogt et al. 1986). However, the largest new soil C contributor over the full 3-year fumigation period was mycorrhizal hyphae (Godbold et al., unpublished results).

Despite the positive effect of FACE on the accumulation of *C*\(_{act}\), into the soil in the third year, the increase in the total soil C pool was smaller in the FACE plots than in the control plots. Hoosbeek et al. (2004) suggested that these opposing effects were caused by a priming effect of the newly incorporated root litter. The priming effect was defined as the stimulation of SOM decomposition caused by the addition of labile substrates (Dalenberg and Jager 1989, Cheng 1999). Through extrapolations from other experiments (e.g., Fox and Comerford 1990, DeLucia et al. 1997, Jones et al. 1998), Hoosbeek et al. (2004) suggested that FACE induced an increase in the fraction of low molecular weight C components in the soil. In contrast to, for example, the Duke FACE site, the extra and more easily degradable C may increase the growth of the microbial populations in the fertile soil of the POPFACE site (Jenkinson and Rayner 1977, Van Veen et al. 1984, Dalenberg and Jager 1989). The soil microbial biomass did not increase in response to the FACE treatment, and much uncertainty exists about microbial biomass responses to elevated [CO\(_2\)] (Larson et al. 2002, Pendall et al. 2004). The microbial quotient, however, was significantly increased by the FACE treatment, which means that more C was available for microbial growth (Ander-
son 2003). Soil respiration rates may have been increased in response to FACE because of the priming effect (Hoosbeek et al. 2004). Large increases in soil CO₂ effluxes were observed in the FACE treatment, in both the second and third years (King et al. 2004). Many factors appeared to contribute to FACE stimulation of soil respiration. FACE increased both root biomass (Lukac et al. 2003) and specific root respiration (I.A. Janssens, unpublished results), as well as C input to the soil (Hoosbeek et al. 2004). We estimated tree respiration by subtracting NPP from GPP and obtained values from 638 to 743 g C m⁻² year⁻¹ (control) and 675 to 956 g C m⁻² year⁻¹ (FACE) in the second year, and from 618 to 1091 g C m⁻² year⁻¹ (control) and 508 to 800 g C m⁻² year⁻¹ (FACE) for the three genotypes in the third year. This corresponds to a relative FACE stimulation of 28% (SE = 10%, P = 0.0047) in the second year, and a nonsignificant mean reduction of 16% (SE = 11%, P = 0.0665) in the third year.

The positive effect of FACE on GPP decreased from the second to the third year, whereas the effect on NPP was similar in both years, resulting in higher NPP:GPP ratios in the FACE treatment. At our site, NEP was not significantly enhanced by FACE. In contrast, FACE stimulated NEP of a pine forest by 41% (Hamilton et al. 2002). However, NEP calculated for the pine forest also includes litter accumulation and fine root increments; only a fraction of this labile C is incorporated in the soil (Hamilton et al. 2002). At the POPFACE site, FACE stimulated respiration of soil C more than the incorporation of new C. Our estimate of NEP is derived from pool size estimates at the end of each year. Although there are errors involved in the upscaling of biomass determinations, this method is superior to extrapolation of specific respiration rates (Hamilton et al. 2002). Although soil CO₂ efflux was increased by FACE at our site and our results suggest increased soil C losses in response to FACE, a remaining challenge is to carefully quantify and understand the effect of elevated [CO₂] on soil C losses. Our study plantation site was converted from agricultural use, which may have influenced soil processes, cancelling out the FACE stimulation of NPP. Although 3 years of research in a fast-growing tree plantation captured the aboveground responses for a closed canopy, belowground processes may need longer to stabilize. Therefore, further research should shed light on the longer term C storage capacity, especially that belowground. The lack of real NEP stimulation at our site is critical in assessing the impact of new plantations established to meet Kyoto Protocol climate change commitments. We have shown that there is no evidence that the C storage capacity of these plantations will improve in a CO₂-enriched atmosphere.

In conclusion, the stimulating effect of FACE on NPP was mainly attributed to an increase in size of relatively slow turnover C pools (wood). In contrast, NEP was not significantly increased by FACE, largely because of increased soil C respiration, which Hoosbeek et al. (2004) attributed to the priming effect of newly incorporated root litter.

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