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Research

Genetic components to belowground carbon fluxes in a riparian forest ecosystem: a common garden approach

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Summary

- Soil carbon dioxide (CO_2) efflux is a major component of terrestrial carbon (C) cycles; yet, the demonstration of covariation between overstory tree genetic-based traits and soil C flux remains a major frontier in understanding biological controls over soil C.
- Here, we used a common garden with two native tree species, *Populus fremontii* and *P. angustifolia*, and their naturally occurring hybrids to test the predictability of belowground C fluxes on the basis of taxonomic identity and genetic marker composition of replicated clones of individual genotypes.
- Three patterns emerged: soil CO_2 efflux and ratios of belowground flux to aboveground productivity differ by as much as 50–150% as a result of differences in clone identity and cross type; on the basis of Mantel tests of molecular marker matrices, we found that *c.* 30% of this variation was genetically based, in which genetically similar trees support more similar soil CO_2 efflux under their canopies than do genetically dissimilar trees; and the patterns detected in an experimental garden match observations in the wild, and seem to be unrelated to measured abiotic factors.
- Our findings suggest that the genetic makeup of the plants growing on soil has a significant influence on the release of C from soils to the atmosphere.

Introduction

Because fluxes of carbon (C) from soils to the atmosphere represent some of the largest biologically mediated C fluxes on Earth (Giardina *et al.*, 2005), an understanding of the plant mediation of these fluxes has global relevance for the C cycle. For example, a 10% change in global terrestrial soil CO_2 efflux could double or completely ameliorate anthropogenic CO_2 emissions (Schlesinger & Andrews, 2000). Plants may affect CO_2 efflux from soils by mediation of both autotrophic (e.g. root respiration) and heterotrophic (e.g. litter decomposition) processes. Thus, inter- and intra-specific differences in plant function have the potential to significantly influence soil CO_2 efflux and global C cycles. Although inter-specific and plant diversity influences on soil CO_2 efflux have been widely examined (e.g. Raich & Tufekcioglu, 2000; Johnson *et al.*, 2008; Dias *et al.*, 2010; Metcalfe *et al.*, 2011), intra-specific influences have only been acknowledged recently (see Fischer *et al.*, 2007).

Forest trees have been increasingly recognized as drivers of soil CO_2 efflux in terrestrial forest ecosystems (Högberg *et al.*, 2001; Janssens *et al.*, 2001; Dias *et al.*, 2010; Högberg, 2010). Although traditionally, respiration from heterotrophic organisms in soils (primarily microbial respiration) has been emphasized, more recent analyses have suggested that autotrophic activity below ground (primarily plant root respiration) may dominate net soil CO_2 efflux from terrestrial forest ecosystems. New techniques have allowed for the quantification of the amount of net soil CO_2 efflux that can be attributable to both sources, and direct autotrophic respiration may account for as much as 40–60% of net soil CO_2 efflux (Högberg *et al.*, 2001; Janssens *et al.*, 2001; Bond-Lamberty *et al.*, 2004; Högberg, 2010). Autotrophic organisms further influence soil CO_2 efflux indirectly through afterlife effects of rhizospheric exudates (Kuzyakov, 2002), rhizospheric priming effects on soil C (Bader & Cheng, 2007; Cheng, 2009), controls on root and leaf turnover, and decomposition (Bowden *et al.*, 1993; Metcalfe *et al.*, 2011). Regardless of heterotrophic vs autotrophic CO_2 sources, plant species can influence substantially bulk C transfer rates to the atmosphere, a phenomenon with clear linkages to global climate change and global C budgets (Schlesinger & Andrews, 2000; Giardina *et al.*, 2005).

Both autotrophic and heterotrophic CO_2 efflux from soils are probably sensitive to plant genetic variation, which can affect the quantity and quality of plant organic matter (e.g. Lojewski *et al.*,

2009), exudates (Phillips *et al.*, 2003) and litter (Schweitzer *et al.*, 2004; LeRoy *et al.*, 2007). Understanding the genetic basis to CO_2 flux is important because genetic sources of variation are generally not included in ecosystem C flux models, genetic variation implies that selection processes could affect ecosystem C flux and this knowledge may assist our understanding of how plant breeding can interact with ecosystem C cycles. Nevertheless, our understanding of plant genetic influences on net soil CO_2 efflux in natural systems is limited (see Fischer *et al.*, 2007). Few studies have attempted to quantify the effect of genetic variation in a common tree on belowground C fluxes in forested ecosystems (but see Rae *et al.*, 2004, 2007). Well-understood genetic-based patterns in aboveground productivity should be expressed below ground when belowground growth is coupled to aboveground growth, particularly as net soil CO_2 efflux can be driven so strongly by autotrophic sources (Högberg, 2010). For example, more productive genotypes probably have higher soil CO_2 efflux beneath their canopies.

One model system for the investigation of plant genetic effects on soil C flux is *Populus* in the Intermountain West, USA (Whitham *et al.*, 2012). Fischer *et al.* (2006) showed a relationship between genetic molecular markers and fine root production of *Populus fremontii*, *P. angustifolia* and their natural hybrids in a common garden of mature trees. Similarly, Fischer *et al.* (2007) found that natural riparian forest stands dominated by *P. fremontii*, *P. angustifolia* and their hybrids along the Weber River, Utah, differed in fine root production, soil CO_2 efflux and belowground C allocation. Because the flux differences were detected along a hybridization gradient, genetic-based differences among tree genotypes were implicated as a major causal agent (Fischer *et al.*, 2007). Further, Lojewski *et al.* (2009) found that tree molecular composition predicted aboveground productivity of trees consistently in natural stands and in four common gardens. The additional heterotrophic contributions to net soil CO_2 efflux are also important, and these fluxes may be related indirectly to tree genetics (e.g. via afterlife effects; Whitham *et al.*, 2012). For instance, Schweitzer *et al.* (2008, 2012) found that soil microbial community structure in the same stands was predictable by genotype. Similarly, Madritch & Hunter (2002, 2003) and Madritch *et al.* (2006) found genetic-based patterns in leaf decomposition and bulk soil respiration in *Quercus* spp. (oaks) and *Populus tremuloides* (aspen) that was attributed to tree genetic identity and diversity. Recent research has also investigated the molecular basis of biomass allocation in *Populus*, *Pinus* and *Eucalyptus* spp. (Li *et al.*, 1991; Retzlaff *et al.*, 2001; Ngugi *et al.*, 2003; Rae *et al.*, 2004, 2007; Wu *et al.*, 2004; Wullschleger *et al.*, 2005). For example, Wullschleger *et al.* (2005) identified 31 quantitative trait loci (QTLs) associated with whole-tree biomass C allocation in synthetically crossed juvenile *Populus* trees. Although this work collectively represents advances in the understanding of root mass pools (Wullschleger *et al.*, 2005) and tree–soil–microbial linkages, this work has not been extended to an understanding of integrated annual belowground tree C fluxes in natural systems.

Here, we build on previous studies in the wild (Fischer *et al.*, 2007), utilizing a common garden environment to explore the genetic-based variation and molecular predictability of belowground C flux among two riparian tree species (*Populus* spp.) and their natural hybrids (collectively referred to as cross types). We hypothesized that, in a common garden, belowground C flux under tree canopies would differ in a manner that would be predictable on the basis of tree molecular variation. We also predicted that our findings would be consistent with patterns identified in the wild (Fischer *et al.*, 2007). Specifically, we hypothesized: higher soil CO_2 efflux in the lowland species *P. fremontii* relative to *P. angustifolia* and their hybrids; that soil CO_2 efflux would vary among genotypes within each species and hybrid cross type; and that across the hybridization gradient, more genetically similar trees would have similar C fluxes than genetically dissimilar trees.

Materials and Methods

Study site

We examined patterns of CO_2 efflux under canopies of 20 genotypes represented by three *Populus fremontii* S. Wats., five *P. angustifolia* James, six natural F_1 hybrids and six backcross hybrid genotypes in a common garden study. All genotypes were replicated three to four times, except for one backcross hybrid genotype that was replicated only twice, resulting in a total of 68 trees used in the entire study. The common garden consists of randomly selected stock from a hybridizing complex of native trees along the Weber River, UT (USA), collected and randomly planted in 1991. Random placement of trees (including all replicates) on a 5×5 m^2 spacing grid within the garden ensured that there was no systematic site bias or spatial autocorrelations that might affect the response variables. This spacing resulted in a *c.* 25-m^2 average growth footprint for each tree. The 15-yr-old common garden has been used previously in studies of variation in tree aboveground productivity (Lojewski *et al.*, 2009), whole-tree sap flux (Fischer *et al.*, 2004), fine root production (Fischer *et al.*, 2006) and heritability of microbial communities and net rates of nitrogen (N) mineralization under *Populus* tree canopies (Schweitzer *et al.*, 2012). Trees range in size from 2.5 to 44 cm in diameter at 1.4 m height, and from 2.9 to 19 m tall. The common garden is located in a historically forested side-drainage of the Weber River (elevation, 1300 m; 41°15′N, 112°00′W). The soil at the common garden is a member of the United States Department of Agriculture Soil Taxonomic family of coarse-loamy, mixed, superactive, mesic Oxyaquic Haploxerolls. The mean annual air temperature of the garden is 10.7°C and the mean annual precipitation is *c.* 468 mm (Ogden Sugar Factory weather station; 3 km southwest of the study site; elevation, 1300 m; http://www.wrcc.dri.edu/summary/climsmslc.html accessed 20 August 2007; period of record, 1 January 1924 to 31 December 2005).

Earlier molecular genetic characterization of genotypes has been extensively described in articles by Keim *et al.* (1989) and Martinsen *et al.* (2001). Briefly, trees were genetically characterized using 97 restriction fragment length polymorphism (RFLP) molecular markers, with 35 markers specific to *P. fremontii*. The

presence or absence of these 35 markers allowed the characterization of relatedness to *P. fremontii*, and the analysis of the entire set of 97 markers allowed the characterization of molecular similarity among genets.

Soil CO_2 efflux

We measured soil CO_2 efflux under individual trees with a LI-COR 6200 (LI-COR, Lincoln, NE, USA) infrared gas analyzer (IRGA) attached to a chamber (11.9 l; diameter, 27.5 cm; height, 20 cm; *sensu* Fischer *et al.*, 2007) with a pressure equalization coil constructed of looped (×3) 1-cm Nalgene™ (ThermoFisher Scientific Inc., Rochecter, NY, USA) tubing, which uses diffusional constraints to prevent CO_2 exchange with the atmosphere. Measurements were taken once a month at midday (between 10:00 and 14:00 h) for 180 s (change in $[CO_2]$ in the headspace was linear over time during this period) during the growing season (May–September 2005) and periodically throughout the winter (October–May 2005/6). The IRGA was calibrated in the field before each measurement period in a similar manner to a procedure used by Hart & DiSalvo (2005). Soil CO_2 efflux measurements were taken on the north side of each tree within 1 m of the tree bole inside a 30-cm-diameter polyvinyl chloride (PVC) soil collar. The PVC soil collar served as a placeholder for measurements and minimized lateral CO_2 diffusion, but otherwise only rested on top of the mineral soil. The collars were incubated in place for *c.* 1 month before measurements. In June, July, August and September in 2005 and July in 2006, measurements were taken on the same five randomly selected trees at 06:00, 12:00, 18:00 and 24:00 h to assess diel variation in soil CO_2 efflux. We found no significant diel variation (full model repeated measures ANOVA with month and time of day as main effects: $F_{3,14} = 1.84$, $P = 0.35$), similar to the finding of another study in natural stands of *Populus* along the Weber River (Fischer *et al.*, 2007). Hence, measurements were considered to be representative of daily soil CO_2 efflux rates and were scaled to annual CO_2 efflux rates by multiplicatively scaling estimates between measurement periods (*sensu* Fischer *et al.*, 2007). During each soil CO_2 efflux measurement, both the soil temperature at a mineral soil depth of 7.5 cm and the air temperature were measured within the chamber. One mineral soil core (depth, 0–7.5 cm; diameter, 1.9 cm) was taken with an Oakfield soil sampler within 0.25 m outside of the soil collar for the determination of gravimetric soil moisture (105°C for 48 h) during each CO_2 efflux sampling.

Tree biomass and productivity measurements

In order to place soil CO_2 efflux patterns in the context of aboveground growth, we estimated the aboveground net primary productivity (ANPP) for all individual trees. The aboveground biomass increment and foliar biomass production were calculated on an individual tree basis using repeated measures of diameter at breast height (DBH; 1.4 m above the base) and locally derived allometric equations for stem increment and foliar biomass (see Lojewski *et al.*, 2009). Tree DBH was measured over 4 yr in May 2003, November 2005 and November 2006. We assumed that negligible DBH growth occurred for the 2003 growing season before May 2003 (before full leaf-out). The mean ANPP (kg m^{-2} yr^{-1}) over the 2003–2006 period was calculated using the following equation:

$$\mathrm{ANPP} = \frac{(B_{2006} - B_{2003}) + (FB_{2003} \times 2) + FB_{2005} + FB_{2006}}{4 * 25\ \mathrm{m}^2}, \quad \text{Eqn 1}$$

(*B* and *FB*, aboveground woody biomass and foliar biomass, respectively, of individual trees for a given year). The footprint (m^2) of each tree was estimated equally for all trees at 25 m^2 on the basis of the original planting footprint of each tree (5-m centers). We did not have measurements of tree foliar biomass from the end of 2003 or 2004, and so we substituted the May 2003 estimates of foliar biomass for both of these periods. This approach does not account for resorption prior to leaf abscission or twig replacement, and therefore our data should be interpreted with caution, as failure to account for resorption could result in overestimates, and failure to account for twig mass could result in underestimates, of foliar and branch NPP by as much as 10–15% (see Hart & DiSalvo, 2005). Throughout, foliar production was estimated through allometry by calculating the foliar biomass from the DBH measurement.

Litterfall inputs to soils

We also measured directly litterfall inputs to soils to further place soil CO_2 efflux in the context of aboveground inputs. Litterfall was captured in a 28.5-cm-diameter littertrap placed within 0.5 m of the PVC soil collars (see the previous section on Soil CO_2 efflux) at the same distance from the tree bole. The littertrap was elevated by *c.* 0.5 m above the ground and constructed of a plastic bucket with water drain holes. Litterfall was collected monthly between July and November in 2005 and 2006 (littertraps were initially placed in June 2005). Negligible litterfall occurred between November 2005 and July 2006, indicating that the majority of litter fall occurs between late summer (when some drought-induced leaf shedding occurs) and November. The litter was dried at 70°C for 72 h prior to weighing.

An index of belowground C allocation

We modified an existing approach for the estimation of belowground C allocation in forests at the stand level using litterfall and soil CO_2 efflux (total belowground C allocation, TBCA; see Raich & Nadelhoffer, 1989) and applied it to individual trees within our experimental common garden (hereafter $TBCA_{single\text{-}tree}$). The classic approach recognizes the relationship of soil CO_2 efflux to inputs from above- and belowground sources, and uses the subtraction of aboveground inputs to estimate the belowground plant contributions to soil CO_2 efflux. Our modification required us to address several potentially confounding assumptions (see later). Although our approach is mechanistically similar to the estimation of TBCA, we emphasize

that this was not directly comparable with ecosystem-scale estimates of TBCA because our sampling approach was limited to under individual tree canopies. Nevertheless, this index should provide a useful measure of comparative belowground C flux at the single-tree scale within our study.

We calculated $TBCA_{single\text{-}tree}$ for individual trees on the basis of the mass balance approach (Raich & Nadelhoffer, 1989; Giardina & Ryan, 2002). The following equation is traditionally used to estimate TBCA:

$$TBCA = F_S + F_E - F_A + \Delta C_S + \Delta C_R + \Delta C_L, \quad \text{Eqn 2}$$

(F_S, soil CO_2 efflux; F_E, C transported out of the soil by leaching; F_A, litterfall; ΔC_S, change in mineral soil C content; ΔC_R, change in C stored in plant roots; ΔC_L, change in the C content of the soil O horizon). In our approach, the scaling of these measures to individual trees required us to address some necessary, but potentially confounding, assumptions. We address the assumptions below with a series of additional measurements. All measures were scaled to the original planting spacing of each tree (25 m^2) in order to place measures within a similar spatial context.

In our approach, we relied primarily on litterfall collection (F_A), allometric estimates of changes in the coarse root pool (ΔC_R) and soil CO_2 efflux measurements (F_S) under individual trees (not randomly distributed throughout the ecosystem). Thus, our effective modified equation was: $TBCA_{single\text{-}tree} = F_S - F_A + \Delta C_R$. In order for this approach to reflect belowground C allocation, the fluxes F_E, ΔC_S, ΔC_R and ΔC_L need to be negligible, in steady state or estimated from aboveground measurements. Over a single year in our relatively arid system, we assumed that F_E was negligible. Similarly, we assumed that ΔC_S was negligible because changes in mineral soil C pools are generally small relative to soil CO_2 efflux and litterfall C (see Giardina & Ryan, 2002; Giardina *et al.*, 2005). In particular, because soil CO_2 efflux values in relatively young systems like ours are so high, departures from steady state on an annual basis are probably small relative to flux values. We measured C_S in September 2006 to obtain a one-time estimate of soil C pools. A soil core (diameter, 5 cm; depth, 15 cm) was taken within 0.5 m on the north side of each tree to determine soil total C and N. Air-dried soil was passed through a 2-mm sieve, and ground to a fine powder. Thirty milligrams of ground soil were weighed into tin capsules and analyzed on a Delta Plus Advantage Elemental Analyzer (Isomass, Inc., Calgary, AB, Canada). Coarse root biomass was assumed to be 25% of aboveground biomass annually (Li *et al.*, 2003; Wullschleger *et al.*, 2005) and was used to calculate ΔC_R; whole-tree C_R was divided by 25 m^2 (based on tree spacing) to achieve a C_R estimate per square meter at each time period. Fine root biomass was assumed to be in steady state during the time of our study and was not included in ΔC_R (annual fine root turnover at the site has also been measured to be close to 1.0 by Fischer *et al.*, 2006). Finally, we assumed that ΔC_L was approximately at steady state ($\Delta C_L = 0$) over a year-long time frame based on the age of the common garden (mature at 14 yr old at the start of this study), the rapid rates of *Populus* litter decomposition (Schweitzer *et al.*, 2004) and the generally closed-canopy structure of the garden (Olson, 1963). The ratio of $TBCA_{single\text{-}tree}$: ANPP was also calculated to provide a comparative index of belowground C allocation (see Litton *et al.*, 2007) vs aboveground productivity (i.e. belowground allocation : aboveground growth).

In order to adapt the TBCA method for use on individual trees, all CO_2 respired by the soil must also be assumed to originate from C fixed by the subject tree. In reality, roots from adjacent trees are likely to occur under the subject tree because of the relatively close proximity of the trees (5×5 m^2 spacing) and their rapid growth rates. To evaluate the potential magnitude of mixing of fine roots from adjacent trees, we randomly selected 10 pairs of trees (20 trees paired by 10 genotypes). On one tree in each genotype pair, we dug a 30-cm-deep trench equidistant (*c.* 2.5 m) between the subject tree bole and an adjacent tree bole in July 2002, and left the corresponding paired genotype tree with no trench. The trenches were double-lined with 0.15-mm-thick plastic and backfilled with soil. For both trenched and untrenched trees, we measured soil CO_2 efflux *c.* 0.5 m from the tree bole facing the adjacent neighboring tree in July 2006. We then compared the difference between trenched and untrenched soil CO_2 efflux as an estimate of the magnitude of root mixing. This approach assumes that differences in soil CO_2 efflux will be caused by differences in root mixing, unaffected by the decay of roots severed in 2002. This may be a source of error and therefore the results should be interpreted with caution. We also tested for effects of direction and distance from the tree bole on soil CO_2 efflux during the July 2006 measurement period. Soil CO_2 efflux was measured 0.5 m from the bole of 20 randomly selected trees in each of the four cardinal directions in order to equally replicate distance intervals from trees four times. In addition, soil CO_2 efflux was measured on 22 randomly selected trees at 0.5 and 1.5 m from the tree bole in the direction of a neighboring tree.

Tests of assumptions

We used a blocked analysis of covariance (ANCOVA) to evaluate the effect of trenching on soil CO_2 efflux, and hence to determine the impact of fine root mixing between neighboring trees on this flux. The trenched and untrenched tree pairs were considered as the blocking factor, trench presence or absence as the main effect, aboveground tree biomass of the measurement tree as the covariate and soil CO_2 efflux as the response variable. With this statistical model, we were able to quantify the relative difference in soil CO_2 efflux caused by trenching without the confounding effect of tree size. Significant differences in soil CO_2 efflux between trenched and untrenched tree pairs were assumed to be caused by root mixing between adjacent trees.

A randomized block ANOVA (n = 20 trees) was used to evaluate the effect of the cardinal direction on the soil CO_2 efflux; each tree was treated as a block and the cardinal direction was the main effect. We used an ANCOVA (n = 22 trees), with distance from the bole as the main effect and tree aboveground biomass as the covariate, to assess the effect of distance from the tree bole on soil CO_2 efflux.

Statistical analysis

We tested the prediction that CO_2 fluxes (including soil CO_2 efflux, the index $TBCA_{single\text{-}tree}$ and $TBCA_{single\text{-}tree}$: ANPP) would vary among cross types using mixed effect models and restricted maximum likelihood (REML), employing the statistics program SAS JMP version 9.0 (SAS Institute, Cary, NC, USA). To determine whether genetic variation and hybridization impacted belowground C dynamics, we constructed mixed models that included hybrid cross type as a fixed effect and replicate, genotype and genotype nested within cross type as random effects. The test statistic for genotype-level effects was determined by likelihood ratio tests, in which the difference between the likelihood ratio of the model described above and the same model with genotype removed was used as a chi-squared value (one-tailed chi-squared distribution, df = 1). Tukey's honestly significant difference (HSD) multiple comparison tests were conducted to compare means among cross types. Estimates of ANPP, $TBCA_{single\text{-}tree}$ and $TBCA_{single\text{-}tree}$: ANPP were natural log transformed to normalize the data. All data are presented as untransformed data in the figures, but we report statistics from the natural log-transformed data.

Mantel tests were used to test the hypothesis that genetically similar trees (across all 20 genotypes) would exhibit similar soil CO_2 efflux, $TBCA_{single\text{-}tree}$ and $TBCA_{single\text{-}tree}$: ANPP ratios. The method used was similar to the widely used approach described in Bangert *et al.* (2006a), Barbour *et al.* (2009) and Zytynska *et al.* (2011). Briefly, we computed distance matrices for the 97 RFLP marker genetic data and related this distance matrix to those created for soil CO_2 efflux, $TBCA_{single\text{-}tree}$ and the $TBCA_{single\text{-}tree}$: ANPP ratios. Distance matrices were computed using Euclidean distances. Mantel tests were performed employing the R Package for multivariate and spatial analysis (Version 4.06d; http://www.bio.umontreal.ca/Casgrain/en/labo/R/v4/index.html; accessed 20 August 2007) and PC-ORD for Windows (version 5.10; MjM Software, Gleneden Beach, OR, USA) with a Monte Carlo randomization method using 1000 permutations. Results from these tests are visually represented using scatter plots of mean genetic distance vs mean phenotype distance of soil CO_2 efflux, $TBCA_{single\text{-}tree}$ or the $TBCA_{single\text{-}tree}$: ANPP ratio (*sensu* Bangert *et al.*, 2006b).

We used ANOVA to determine whether tree cross type (a categorical variable) explained the variation in mineral soil moisture and temperature over the timeframe of this study. The statistical software SAS JMP version 9.0 (SAS Institute) was used for all ANOVAs, ANCOVAs and regressions. An alpha level of 0.05 was used to determine statistical significance in all analyses.

Results

Cross type and genotype effects on C flux

We found significant plant genetic effects on associated soil CO_2 efflux, $TBCA_{single\text{-}tree}$ and the $TBCA_{single\text{-}tree}$: ANPP ratio. ANOVA indicated that cross type (Fig. 1) impacted significantly on soil CO_2 efflux ($F_{3,52}$ = 5.104, P = 0.01), $TBCA_{single\text{-}tree}$ ($F_{3,52}$ = 8.05, P = 0.002) and the $TBCA_{single\text{-}tree}$: ANPP ratio ($F_{3,52}$ = 10.44, P < 0.001). Tukey's HSD multiple comparison test showed that *P. fremontii* and F_1 hybrids generally had higher

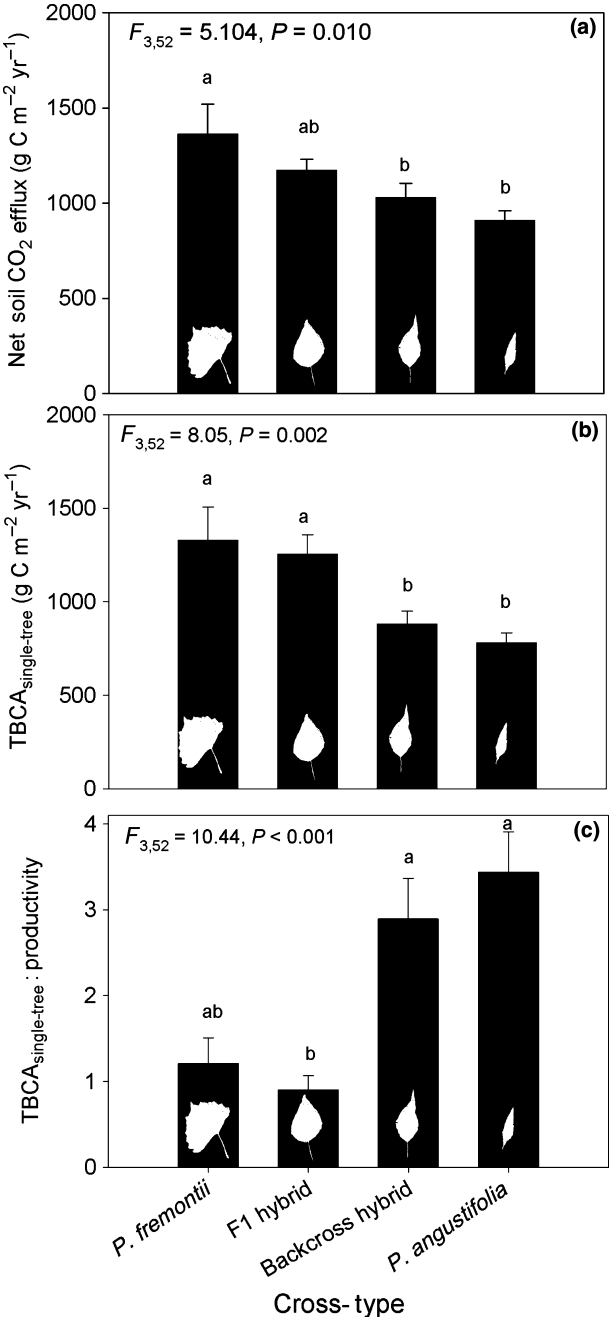

Fig. 1 Mean carbon (C) fluxes and whole-tree C allocation of *Populus fremontii*, *P. angustifolia* and their natural F_1 and backcross hybrids grown in a common garden in Ogden, UT, USA. (a) Soil CO_2 efflux, (b) total belowground carbon allocation ($TBCA_{single\text{-}tree}$) and (c) $TBCA_{single\text{-}tree}$: aboveground net primary productivity (ANPP) ratio. Different letters denote significant differences from Tukey's honestly significant difference (HSD) multiple comparison test at P < 0.05. Error bars represent one 95% confidence interval (CI) of the mean. Statistical analyses were performed on natural log-transformed data, but untransformed data are shown.

belowground C fluxes, but lower $TBCA_{single\text{-}tree}$: ANPP ratios, than backcross hybrids and *P. angustifolia*. Although *P. fremontii* and F_1 hybrids were sometimes intermediate in each pattern, the backcross hybrids and *P. angustifolia* were often similar, and differentiated statistically from *P. fremontii*, F_1 hybrids or both. We found no significant difference among cross types in soil moisture ($F_{3,832} = 0.85$, $P = 0.46$) or soil temperature ($F_{3,832} = 0.04$, $P = 0.99$), suggesting that plant genetic factors did not predictably result in altered soil moisture and temperature under tree canopies.

At the genotype level within cross types, results were mixed. We did not detect significant differences in net soil CO_2 efflux ($\chi^2 = 0.16$, $P = 0.35$) or $TBCA_{single\text{-}tree}$ ($\chi^2 = 0.11$ $P = 0.37$). However, for the ratio $TBCA_{single\text{-}tree}$: ANPP, we found significant differences among genotypes ($\chi^2 = 3.62$, $P = 0.028$). Nevertheless, low replication at the genotype level may have made the detection of the significance of genotype more difficult, and analyses using marker distributions instead of individual genotype identities may provide a better illustration of the role of genetic variation in controlling these fluxes.

Accordingly, Mantel tests across all cross types revealed that genetically similar trees (based on all 97 molecular markers) were also similar with respect to soil CO_2 efflux, $TBCA_{single\text{-}tree}$ and $TBCA_{single\text{-}tree}$: ANPP ratio (Mantel $r = 0.232$, $P = 0.005$, Mantel $r = 0.363$, $P < 0.001$ and Mantel $r = 0.259$, $P = 0.009$, respectively; Fig. 2). This result suggests that genetically more similar genotypes also had more similar efflux traits, suggesting additive responses in C flux to genetic variation. Because these Mantel tests analyze fluxes as a function of a continuous trait (i.e. genetic distance between trees), rather than as discrete categories of pure and hybrid cross types, the linear relationships in Fig. 2 suggest that fluxes behave like continuous traits that vary predictably across the genetic continuum. Nevertheless, this pattern may have been driven by genetic differences among the two parent species (*P. fremontii* and *P. angustifolia*). A similar Mantel analysis including only the hybrid types was also performed to examine how genetic similarity was related to C flux when the parental species were excluded from the analysis. In this analysis, we found mixed, but often insignificant, patterns of genetic similarity relationships with C flux traits. Genetic similarity was related to soil CO_2 efflux (Mantel $r = 0.324$, $P = 0.036$), but not to $TBCA_{single\text{-}tree}$ (Mantel $r = 0.110$, $P = 0.183$) or $TBCA_{single\text{-}tree}$: ANPP (Mantel $r = -0.099$, $P = 0.217$). Similar Mantel tests within parental species were not possible because there was limited variation in molecular markers and limited genotype replication in the parental species.

We found no significant effect of trenching on soil CO_2 efflux ($F_{11,19} = 0.76$, $P = 0.67$), suggesting that the impact of root mixing by adjacent trees on soil CO_2 efflux was not large. Nevertheless, root mixing from adjacent trees may still be a source of error and results should be interpreted accordingly. Although variation caused by undetected root mixing among adjacent trees was possible in this study, the results described above suggest that, despite this potential error, tree identity still explained a significant amount of variation in soil CO_2 efflux and our index of TBCA (Figs 1a,b, 2a,b).

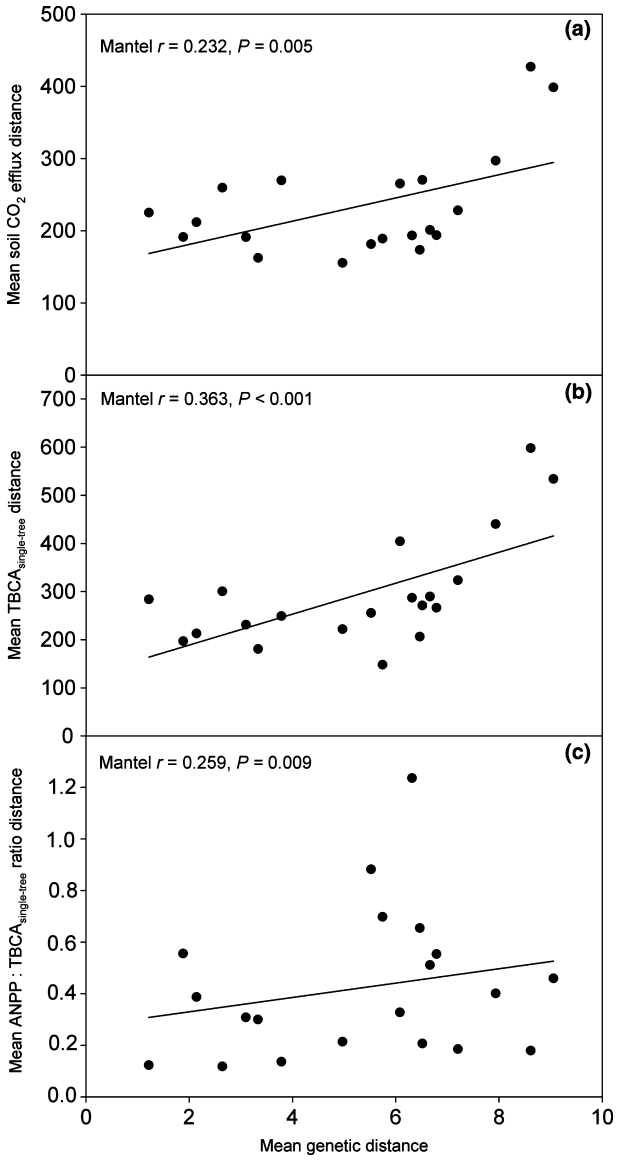

Fig. 2 Comparison of average distance in tree molecular genetic composition of 20 *Populus* genotypes, from a common garden in Ogden, UT, USA, to phenotypic distance in (a) soil CO_2 efflux, (b) total belowground carbon allocation ($TBCA_{single\text{-}tree}$) and (c) the ratio of $TBCA_{single\text{-}tree}$: aboveground net primary productivity (ANPP) as a visualization of Mantel tests. Higher numbers on the horizontal and vertical axes denote greater genetic and phenotypic differences between genotypes. Molecular genetic composition was calculated from 97 random restriction fragment length polymorphism markers.

We also found no significant effect of direction ($F_{3,56} = 1.75$, $P = 0.17$) or distance from the tree ($F_{2,41} = 2.55$, $P = 0.20$) on soil CO_2 efflux. This lack of a significant effect of direction or distance is consistent with the hypothesis that our measurement of soil CO_2 efflux was not predictably biased by factors related to azimuth or distance from the base of the tree within the garden. Nevertheless, undetectable patterns associated with azimuth and distance from the tree may be sources or error in the study, and therefore should be interpreted with caution.

Discussion

Our data suggest that genetic differences at the cross type level across a hybridizing complex can have large effects on belowground ecosystem C flux. Although patterns in net soil CO_2 efflux represent patterns attributable to both autotrophic and heterotrophic respiration, patterns in our index of $TBCA_{single\text{-}tree}$ may be the result of belowground C flux in the form of root growth, root respiration and complex rhizosphere fluxes (including mycorrhizae). For both soil CO_2 efflux and $TBCA_{single\text{-}tree}$, our findings broadly support predictable genetic-based patterns in belowground C fluxes previously found in natural *Populus* stands (Fischer *et al.*, 2007). Soil CO_2 efflux and belowground C allocation were higher in low-elevation *P. fremontii*, but high-elevation *P. angustifolia* exhibited much higher fluxes proportional to aboveground productivity. This pattern in proportional allocation below ground was similar to patterns in fine root production described in the same system (Fischer *et al.*, 2006, 2007). Because our data were generated from within a common garden environment, they suggest that patterns in the wild (Fischer *et al.*, 2007) are reflective of genetic-based mechanisms that are stable in a common environment (*sensu* Lojewski *et al.*, 2009). Our Mantel analyses also suggest that C flux below ground can be predicted on the basis of molecular similarities in trees. These similarities were most easily detected with the genetic differences between species, but significant and weaker effects were found among genotypes within hybrid cross types. Overall, genetic predictability of soil C fluxes was measurable, with soils under trees with a similar molecular composition (across all cross types) displaying similarity in C exchange with the atmosphere.

Our data for soil CO_2 efflux demonstrate high and variable rates of soil CO_2 efflux in our system. For example, the variation in average soil CO_2 efflux in our study, ranging from *c.* 900 to 1400 g m^{-2} yr^{-1}, is roughly equivalent to differences between soil CO_2 efflux in northern temperate vs tropical systems in a recent review (Davidson *et al.*, 2002). The high values in our study are reflective of the high productivity in these riparian systems characterized by fast growth in riparian trees. The magnitude of the differences highlights that differences in soil CO_2 efflux based on plant overstory genetics are not only detectable, but may also be large.

Recent work in other natural ecosystems has similarly demonstrated patterns in leaf decomposition and CO_2 fluxes that suggest that tree genetic-based regulation of soil C flux may be widespread. Madritch & Hunter (2002, 2003) found differences in leaf decomposition and soil CO_2 efflux that were predictable on the basis of oak leaf phenotype from mixed *Quercus* spp. (oak) forest stands in Georgia, USA. Intra-generic differences in *Quercus* spp. fine root morphology and longevity in soils (Espeleta *et al.*, 2009) may also be related to differences in soil CO_2 efflux at the intra-generic scale. Similarly, Madritch *et al.* (2006) demonstrated differences in leaf decomposition and C release to soils which were consistent with *P. tremuloides* (quaking aspen) genotype identities in natural clonal stands in Wisconsin, USA. Finally, genetic-based differences in C flux have also been found in sea grass-dominated systems (Hughes *et al.*, 2008, 2009; Tomas *et al.*, 2011) and in herbaceous angiosperms (Crutsinger *et al.*, 2006, 2009).

Predictable variation in aboveground C flux occurs in the *Populus* forests studied across a range of common gardens at different elevations and microclimates (Lojewski *et al.*, 2009). If soil CO_2 efflux patterns are autotrophically driven, higher soil CO_2 efflux could be the result of higher aboveground productivity (i.e. larger trees respire more below ground; Lojewski *et al.*, 2009), or the cause of aboveground differences in productivity when some genotypes exhibit more advantageous rooting below ground (also see Fischer *et al.*, 2006). Although our study cannot immediately distinguish between this potential cause or effect relationship, our work highlights the need for future studies spanning multiple common gardens, and integrating above- and belowground C flux patterns. Interestingly, our data suggest a switch in which, despite higher overall net soil CO_2 efflux and $TBCA_{single\text{-}tree}$ in two cross types, these same cross types are lowest in $TBCA_{single\text{-}tree}$ proportional to aboveground productivity. This finding is consistent with potential indirect mechanisms, such as feedbacks on C allocation associated with foliar tannin effects on soils (Fischer *et al.*, 2006, 2007; Pregitzer *et al.*, 2010; Smith *et al.*, 2012). For example, foliar tannin-induced decreases in nutrient availability (Rice & Pancholy, 1973; Northup *et al.*, 1995; Hättenschwiler & Vitousek, 2000; Schweitzer *et al.*, 2004) may result in higher belowground investment in trees, as shown in Fig. 2(c), where *P. angustifolia* and backcross types (known to be higher in foliar tannins compared with other cross types; Schweitzer *et al.*, 2004) demonstrate higher proportional belowground C allocation. These mechanisms may warrant future research, especially based on the extensive previous research on this topic in cottonwood forests (see Schweitzer *et al.*, 2004; Fischer *et al.*, 2006, 2007; Lojewski *et al.*, 2009).

It is important to highlight that our results for net soil CO_2 efflux reflect the influence of both autotrophic and heterotrophic CO_2 release. Although our study cannot directly disentangle autotrophic and heterotrophic soil CO_2, future studies on plant genetic effects should address differences in these CO_2 sources. Previous work has demonstrated that genetic differences in secondary plant chemicals within and among *Populus* cross types predict microbial soil communities (Schweitzer *et al.*, 2008, 2012), with implications for heterotrophic soil CO_2 efflux. Autotrophic and heterotrophic CO_2 releases from soils have dramatically different implications for global C cycles. Because heterotrophic C is largely the result of the decomposition of C in soil and detritus pools, high heterotrophic C release may decrease soil C storage, whereas autotrophic C efflux below ground may represent the flux of recently fixed photosynthate associated with respiration (Högberg, 2010). Our findings suggest the possibility for re-enforcing patterns in heterotrophic and autotrophic belowground CO_2 efflux, and emphasize that genetic differences among trees can play a large role in determining bulk CO_2 transfer to the atmosphere from soils via multiple pathways.

A growing body of work demonstrating the genetic regulation of C pools (Retzlaff *et al.*, 2001; Ngugi *et al.*, 2003; Wu *et al.*, 2004; Wullschleger *et al.*, 2005) has led to interest in the use of woody species for the sequestration of C in terrestrial ecosystems

(Jansson *et al.*, 2010). Our data here suggest that soil C fluxes in natural systems are already sensitive to genetic variation, at least at the cross type scale. In our study, variation in soil CO_2 fluxes appears to be consistent with belowground C allocation, and is predictable on the basis of the molecular composition of genotypes. Future work may help to untangle how these genetic-based patterns are related to potential genetic-based differences in soil C storage.

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