Plant Soil (2008) 308:239–253
DOI 10.1007/s11104-008-9624-2

REGULAR ARTICLE

The influence of soil type and altered lignin biosynthesis on the growth and above and belowground biomass allocation of *Populus tremuloides*

Jessica E. Hancock · Kate L. Bradley · Christian P. Giardina · Kurt S. Pregitzer

Received: 19 November 2007 / Accepted: 10 April 2008 / Published online: 9 May 2008

Abstract Plants influence soil carbon (C) formation through the quality and quantity of C released to soil. Soil type, in turn can modify a plant's influence on soil through effects on plant production, tissue quality and regulation of soil C decomposition and stabilization. Wild-type aspen and three transgenic aspen lines expressing reduced stem lignin concentrations and/or increased syringyl (S) to guaiacyl (G) ratio lignin were grown in greenhouse mesocosms containing a sandy loam, a silt loam, or a clay loam soil for 6 months in order to examine the effects of altered lignin biosynthesis and soil type on biomass partitioning (above vs. belowground) and soil C processes. Results indicated that soil type significantly affected plant performance. Aspen grown in soils with high sand/low clay content accumulated the most total biomass, while aspen grown in soils with high clay content accumulated the least total biomass. These reductions in growth combined with specific soil characteristics led to differences among soil types in soil C formation. Transformed aspen expressing high syringyl/guaiacyl (S/G) lignin accumulated less total plant C and subsequently accumulated less aspen derived C in soil. Reduced lignin content alone in aspen did not affect plant growth or soil C formation. There were significant soil type × genetic line interactions indicating that growth and soil C formation for transgenic and wild type aspen lines varied among the different soil types. Given these interactions, future investigation needs to include long-term field studies across a range of soil types before transgenic aspen are widely planted.

Keywords Biomass allocation · Growth physiology · Plant productivity · Soil carbon formation · Soil texture · syringyl to guaiacyl ratio

Responsible Editor: B. Wick.

J. E. Hancock · K. L. Bradley
Ecosystem Science Center,
School of Forest Resources and Environmental Science,
Michigan Technological University,
1400 Townsend Drive,
Houghton, MI 49931, USA

C. P. Giardina (✉)
PSW Research Station,
Institute of Pacific Islands Forestry-USDA Forest Service,
60 Nowelo Street,
Hilo, HI 96720, USA
e-mail: cgiardina@fs.fed.us

K. S. Pregitzer
Department of Natural Resources, University of Nevada,
1000 Valley Road,
Reno, NV 89512, USA

Introduction

Populus tremuloides (aspen) is one of the most widely distributed native trees in North America (Barnes and Wagner 2004). Aspen is fast growing, easily propagated, and the genus serves as a model for tree molecular biology (Bradshaw et al. 2000; Brunner et al. 2004). The genetic transformation of both lignin

quantity and syringyl/guaiacyl (S/G) lignin can now be achieved in *Populus* spp. (Tsai et al. 1998; Hu et al. 1999; Harding et al. 2002; Li et al. 2003), and aspen with altered lignin biosynthesis, particularly genetic lines with low lignin content and high S/G lignin, could be beneficial to Kraft pulping by reducing economic costs while improving the environmental impacts of the process (Baucher et al. 1996; Chen et al. 2001; Strauss et al. 2001; Pilate et al. 2002; Baucher et al. 2003). Furthermore, manipulation of lignin biosynthesis in *Populus* has provided a useful tool for investigating the potential enhancement of C sequestration by forests, which account for 60% of terrestrial C (Atjay et al. 1979; Jobbagy and Jackson 2000; Herrera 2005; Lorenz and Lal 2005; Pilate et al. 2002; Tilston et al. 2004; Halpin et al. 2007). Most recently, lignin manipulated transgenic aspen have also been used to understand how changes in plant growth and tissue chemistry can affect aspen growth physiology and soil C formation rates (Hancock et al. 2007), as well as composition of soil microbial communities and soil enzyme activity (Bradley et al. 2007).

Lignin, a key structural element in plants, is the second most abundant polymer on Earth, and lignin concentrations in plant litter strongly control their decomposition rates (Meentemeyer 1978; Melillo et al. 1982; Berg and Lundmark 1985; Hobbie et al. 2006). While litter decomposition rates are increasingly well understood (Parton et al. 2007), the relationship between plant properties, such as tissue quality and quantity, and soil C formation and decomposition rates are less well understood (Giardina et al. 2001; Russell et al. 2007).

Similarly there is a poor understanding of how transgenic changes in tissue quality impact plant growth and the allocation of C above and belowground. In the only field study of transgenic aspen, Pilate et al. (2002) found that aspen with reduced lignin content or reduced S/G did not perform differently from control aspen. Pilate et al. (2002) did, however, find that transgenic aspen had significantly higher rates of root decomposition compared to control aspen. Further investigation of coarse wood decomposition from this field trial found no effects of altered lignin biosynthesis on decomposition rates (Tilston et al. 2004).

We have developed a successful greenhouse approach to quantify the effects of altered lignin biosynthesis in aspen on tissue chemistry, growth, above and below biomass allocation and soil C formation (Hancock et al. 2007). We determined that under conditions of non-limiting water or nutrients, reductions in lignin biosynthesis in stem tissue were not translated to leaves or roots (the major source of detrital inputs to soil). However, in contrast to Pilate et al. (2002), we found that altered lignin biosynthesis did significantly affect above and belowground biomass allocation and also reduced root growth in all transgenic lines, particularly in aspen with large increases in syringyl lignin (Hancock et al. 2007). Through the use of ^{13}C isotope techniques and greenhouse mesocosms with soils sampled from C_4 dominated grasslands, we were also able to examine the effects of altered lignin biosynthesis in aspen on soil C formation rates and found that reductions in root growth due to altered lignin biosynthesis corresponded to reductions in aspen-derived soil C (Hancock et al. 2007).

Given the potential effects of lignin modification on plant primary production and soil organic C storage, there is a strong need to continue evaluating the performance and ecological impacts of lignin modified transgenic aspen before these modified trees are widely planted in the field. Soil type may have a significant impact on ecosystem C through its influence on plant growth (Fisher and Binkley 2000; Silver et al. 2000; Bouma and Bryla 2000; Cox et al. 2006). Furthermore, soil type—especially clay mineralogy and content—can control soil C decomposition, formation and stabilization through its physical and structural properties (Sorensen 1972; Christensen 1987; Schimel et al. 1994; Hassink 1997; Torn et al. 1997; Gale et al. 2000; Six et al. 2002). However, we know of no study designed to understand how lignin-modified transgenic aspen may perform in different soil types.

In the current study, we expanded upon our previous greenhouse experiment by growing control and transgenic lines of aspen in three different soil types. Our objectives were to understand the independent and interactive effects of altered lignin biosynthesis and soil type on plant growth and above and belowground biomass partitioning as well as to understand the subsequent effects on soil C formation. In designing this experiment, we developed several hypotheses about the effects of soil type and altered lignin biosynthesis on aspen plant growth (hypothesis 1) and on soil C formation (hypothesis 2). In addition, few studies of lignin-modified aspen have examined

trait stability through micro-propagation and establishment, and so we included two modified lines from our previous experiment to examine the reproducibility of trait expression between experiments.

Although the structural and physical properties of our soils varied greatly (Table 1), we expected that plant growth would not differ between soil types due to an optimal supply of fertilizer and H_2O through regular additions (hypothesis 1a). Based on our previous results, which showed that genetic modifications to lignin biosynthesis reduced belowground biomass in transgenic aspen but not aboveground biomass (Hancock et al. 2007), we anticipated that aboveground biomass would not differ among low lignin lines and control aspen, but that root biomass would be reduced in low lignin lines (hypothesis 1b). Including two lines from the previous experiment allowed us to assess consistency of these trait expressions between experiments and soil types. As part of the test of this hypothesis, we expected the effects of altered lignin on plant growth and C allocation to be constant over time. Second, given that aspen with greatly increased syringyl content had less above and belowground biomass compared to other transgenic lines, we expected that the new high S/G line utilized in the current experiment would also have reduced above and belowground biomass compared to control aspen (hypothesis 1c). While our previous experiment found this to be true for one line of high S/G aspen, we had no basis for extrapolating findings to other high S/G lines.

While we expected that plant growth would be similar across soil types (hypothesis 1a), we hypothesized that soil C formation would be highest in soils with the highest clay contents (clay loam) and lowest in the soil with the highest sand content (sandy loam; hypothesis 2). Finer texture soils with higher clay contents have higher capacity to chemically stabilize organic matter (Stevenson 1994; Hassink 1997; Six et al. 2002), and also to physically protect organic matter through the formation of aggregates (van Veen and Kuikman 1990; Jenkinson 1988). Thus, soils with more clay than sand tend to have slower rates of soil C loss (Bonde et al. 1992; Desjardins et al. 1994). Finally, we previously have demonstrated that soil C formation rates are positively related to total root biomass (Hancock et al. 2007). Therefore, we anticipated that transgenic aspen lines with the lowest root biomass (high S/G line) would have the lowest rates of soil C formation, while aspen with the highest root biomass (control aspen) would have the highest rates of soil C formation.

Table 1 Summary of climatic characteristics, dominant plant species, and soil properties of the three soils used in this study

	Central Plains Experimental Range, Colorado	Konza Prairie, Kansas	Blackland Prairie Temple, Texas
Latitude (N)	40°48′	39°05′	31°6′
Longitude (W)	104°46′	96°35′	97°21′
Ecosystem	Shortgrass steppe	Tallgrass prairie	Tallgrass prairie
Mean annual precipitation (mm)	307	835	877
Mean annual temperature (°C)	8.9	12.8	25.8
Dominant perennial grasses	*Bouteloua gracilis* *Buchloe dactyloides*	*Andropogon gerardii* *Schizachyrium scoparium*	*Bothriochloa ischemum*
Percent sand by mass	73[a]	27[b]	30[c]
Percent silt by mass	16[a]	52[b]	35[c]
Percent clay by mass	11[a]	21[b]	35[c]
pH in water	6.4[a]	7.7[b]	7.9[c]
Percent C by mass	0.9[a]	3.4[b]	2.4[c]
Percent N by mass	0.1[a]	0.3[b]	0.2[c]
Available P (g/kg)	0.227[a]	0.525[b]	0.484[c]
Available K (g/kg)	2.334[a]	7.768[b]	3.967[c]
Available Ca (g/kg)	2.201[a]	13.982[b]	141.444[c]
Available Mg (g/kg)	1.951[a]	7.058[b]	3.974[c]

Means are given for soil physical and chemical properties. Values followed by different letters indicate significant differences by LSD test ($p<0.05$).

Materials and methods

Overview

For 6 months (May 2005–November 2005), we grew a control line of aspen and three transgenic lines derived from the control in three soil types including clay loam, silt loam, and sandy loam textured soils. We used the two low lignin lines described in our previous experiment (lines 23 and 141; Hancock et al. 2007). However, because the high S/G aspen (150% increase in S/G) line from our previous experiment performed poorly, we utilized a new line of aspen that expressed a 60% increase in S/G (line 72). In order to examine the effects of altered lignin biosynthesis and soil type on plant performance we measured maximum photosynthetic rates throughout the summer. Then at harvest, we measured total plant leaf area, height, and above and belowground biomass. Furthermore, by growing aspen (C_3-based photosynthesis) in soils that had supported grasses relying on C_4-photosynthesis, we were able to utilize ^{13}C isotope tracing techniques to understand how altered lignin biosynthesis and soil texture affected soil C formation (Hancock et al. 2007).

Soil collection

We sampled soils from three C_4 dominated grassland sites: the Pawnee National Grassland near the Colorado Central Plains Experimental Range (CPER) northeast of Fort Collins, Colorado; the Konza Prairie Biological Station, Kansas; and the Blackland Prairie at the USDA Grassland Station in Temple, Texas. The climatic conditions and dominant vegetation differ among the three soil types, but these soils were selected for this experiment primarily based on their texture differences (Table 1). The Colorado soil is an Olney series fine sandy loam (US Soil Taxonomy: fine-loamy, mixed, superactive, mesic Ustic Haplargrid). The Kansas soil series is a Reading silt loam (fine-silty, mixed superactive, mesic Pachic Argiudoll). The Texas soil series is Austin silty clay (fine silty, carbonatic, thermic Udorthentic Haplustoll).

In May 2005, approximately 250 kg of mineral soil was collected from each of the grasslands. At each randomly selected sampling site, plants and the surface mineral soil up to 5 cm depth were removed and the underlying soil was sampled to a depth of 30 cm. Soils were immediately shipped to Houghton, MI and each soil type was individually twice-sieved through two offset layers of 4-mm mesh to remove roots and rocks and was then further homogenized through repeated mixing. Any visible remaining roots or detritus were removed by hand during this homogenization step as well as during creation of the mesocosms (see below). We did not construct control mesocosms—that is "soil only" mesocosm without plants. Soils were not sterilized or treated additionally before out-planting several weeks later.

Transgenic aspen

Transgenic trembling aspen (*Populus tremuloides*) lines were generated by *Agrobacterium*-mediated transformation (Tsai et al. 1994, 1998; Ho et al. 1998; Hu et al. 1999). We used a single control aspen line and three transgenic aspen lines derived from this control line (Table 2). Antisense suppression of the gene encoding 4-coumarate-CoA ligase (4CL), resulted in a line with a 35% reduction in stem lignin concentration and an associated 8% increase in stem cellulose concentration (line 23; Hu et al. 1999).

Table 2 Aspen (*Populus tremuloides*) stem tissue chemistry of transgenic and control plants used in the greenhouse trials (From Bradley et al. 2007)

	Line 72	Line 141	Line 23	Line 271 (Control)
Description	40% decrease in lignin, 60% increase in S/G	50% decrease in lignin, 20% increase in S/G	35% decrease in lignin, normal S/G	Normal lignin, normal S/G
Stem percent lignin	13.7	10.7	14.4	22.2
Syringyl/guaiacyl monomers in lignin (S/G ratio)	3.6	2.7	2.2	2.2
Stem percent cellulose	49.2	53.3	44.8	41.4

Over-expression of the gene encoding coniferaldehyde 5-hydroxylase (CAld5H) increases stem syringyl/guaiacyl ratio (Li et al. 2003). Thus, simultaneous suppression of 4CL and over-expression of CAld5H led to lines with a 50% reduction in stem lignin concentrations and a 20% increase in stem lignin S/G (line 141) or a 40% reduction in lignin with a 60% increase in S/G (line 72).

Transgenic and control aspen were micropropagated and regenerated in March 2005. In May 2005, rooted micropropagates were transferred from sterile culture to small containers (~230 g soil) containing one of the three soil types and maintained in mist chambers for 3 weeks. Eight plants per line were repotted in 2.5 l containers filled with one of the three soil types (N=96) and were then transferred to greenhouse benches in June 2005. The greenhouse trial was a full factorial experiment (four genetic lines × three soil types). Aspen were grown under natural light conditions through July, after which (August through November) we supplemented light to maintain a 16-h photoperiod from. Plants were watered daily to soil moisture capacity with an automated irrigation system. Complete fertilizer (NPK + micronutrient; 0.02% B, 0.07% Cu, 0.15% Fe, 0.05% Mn, 0.00005% Mo, 0.06% Zn) was applied at a rate of 0.06 mg N, 0.09 mg P, and 0.06 mg K per plant once a week throughout the experiment. In November 2005, plants were harvested for total above (leaves, stems) and belowground (roots) biomass.

Photosynthesis

Maximum photosynthetic rates (A_{max}) were measured on five plants per genetic line × soil type treatment (N=60) on July 27–31, August 23–26, and September 18–23, 2005. Measurements were made using a LiCor 6400 portable photosynthesis system (LiCor, Lincoln, NE, USA). At least one plant of each treatment was sampled each day, and plants from different treatments were sampled randomly to minimize confounding treatment effects due to variations in weather conditions and diurnal patterns. A_{max} was measured on the youngest fully expanded leaf of each plant. Light was controlled with a red-blue light-emitting diode and light levels were set at a photosynthetic photon flux density of 1,200 μmol m^{-2} s^{-1}. Relative humidity was set at 55–65% by a combination of removing water vapor from incoming air and re-humidification during transpiration. Temperature was not regulated, with measured greenhouse temperatures ranging from 26–31°C during July measurements, 27–29°C during August measurements, and 24–29°C during September measurements.

Plant growth and biomass allocation

Plants were harvested from November 8–16, 2005. One plant per treatment was harvested each day to avoid confounding harvest time and treatment effects. At harvest, tissues were separated into leaves, roots, and stems. At this time, the height and total leaf area of each plant was measured. For total plant leaf area, we measured the leaf area of all leaves with a LiCor 3100C leaf area meter. Individual leaf area was calculated for each plant as the total leaf area divided by the number of leaves per plant. We also calculated specific leaf area (SLA) for each plant as total leaf area at harvest divided by total leaf dry mass.

To quantify root biomass, excess soil was shaken gently off of the root mass, and the root mass and any remaining soil were placed in a de-ionized water bath and washed by hand to completely remove soil from roots. During our single season experiment, our watering regime resulted in plants that generally were not "root-bound", though the largest plants did fully occupy soil with some roots beginning to form more densely on the outside of the containers. All tissues and subsamples were immediately frozen (−80°C), and then lyophilized and weighed to obtain dry weights of each tissue component. Leaves that senesced during the experiment were collected, dried, weighed and added to total leaf biomass. Subsamples of roots from each individual plant were ashed in a countertop furnace at 450°C to determine mineral content. Thus, root biomass is expressed on a mineral-free basis.

Tissue samples were ground through a Wiley Mill and then subsamples were taken and bulked for each genetic line × soil type treatment. Bulked treatment tissue samples were further ground in a ball mill and analyzed for ^{13}C and percent C using an elemental analyzer (Costech 4010, Costech Analytical Technologies, Inc., Valencia, CA, USA) connected to the Isotope Ratio Mass Spectrometer (Delta^{PLUS}, Finnigan MAT, Bremen, Germany) by a Conflo III interface (Finnigan MAT).

Soil carbon formation

Subsamples of soil were collected from each 2.5 l pot initially before trees were transplanted in June and again at harvest in November. Roots were handpicked from soil subsamples to ensure that all visible fine roots were removed. Initial and post-harvest soils were ground and analyzed for percent C and ^{13}C following acid vapor fumigation to remove all carbonates (See Hancock et al. 2007 for details). The percentage soil C derived from aspen plants was estimated using the following equation:

$$\%\ \text{C from } C_3 = (\delta - \delta_0 / \delta_1 - \delta_0) \times 100 \quad (1)$$

where δ is the $\delta^{13}C$ of the soil sample, δ_0 is the initial $\delta^{13}C$ of soil from C_4 grassland, and the δ_1 is the $\delta^{13}C$ of C_3 plant material (aspen roots and leaves). No leaf litter accumulated in the pots during the experiment as all senesced leaf material was immediately collected to estimate aboveground plant production.

Statistical analysis

Two -way ANOVAs, with soil type and genetic line as fixed effects, were used to test for differences in plant growth, biomass allocation, and soil C formation. We also performed one-way ANOVAs to examine the effects of genetic line on plant growth, biomass allocation, and soil C formation within each soil type. All analyses were performed with the GLM univariate procedure in SPSS (SPSS, Inc., Chicago, IL, USA), and an alpha=0.05 was used to protect against type I errors. Where our F-test showed significant differences, we used a Fisher (restricted) least significant difference post hoc test to determine significant differences between treatments.

Results

Plant growth—A_{max}, plant leaf area and height

Maximum photosynthetic rates (A_{max}) peaked in July and declined to the lowest rates in September (Table 3). In July and August, there was a significant effect of genetic line on A_{max} ($p<0.01$), but by September, genetic line effects on A_{max} disappeared. There was no effect of soil type on A_{max} in any month and the genetic line × soil type interaction was not significant. In July, across soil types, line 72 maintained significantly higher rates of A_{max} than line 141 and control aspen (Table 3). In August, A_{max} for the control line was significantly less than the other three lines (Table 3).

Although we did not identify a significant genetic line × soil type interaction for A_{max}, variation was high and so we examined the effect of genetic line on A_{max} within each soil type to identify whether patterns in the overall analysis were consistent across soil types. These additional analyses showed that in the Colorado soil, line 23 maintained the highest rates of A_{max}, while in the Kansas soil, line 72 maintained the highest rates of A_{max} (Table 3). In the Texas soil, there were no effects of genetic line at any date (Table 3).

There was a significant effect of soil type ($p<0.001$) and genetic line ($p<0.001$) on total leaf area and total height, and the genetic line × soil type interaction was significant ($p<0.05$). Total leaf area and height were highest in the Colorado soil and lowest in the Texas soil (Table 4). Across soil types, line 72 had significantly smaller total leaf area and the smallest stature compared to the other three aspen lines (Table 4). Because of a significant genetic line by soil type interaction, we examined the effect of genetic line on height and total leaf area within each soil type and determined that the same trends of genetic line were generally observed within each soil type (Table 4).

There was a significant effect of soil type ($p<0.001$) and genetic line ($p<0.001$) on individual leaf size, but the genetic line × soil type interaction was not significant. Plants in the Colorado soil had the largest individual leaves, while plants in the Texas soil had the smallest individual leaves (Table 4). Despite smaller total leaf area, across soils, line 72 had larger individual leaves compared to lines 141 and 23 (Table 4), but did not differ significantly from control aspen (Table 4). Line 23 had the smallest individual leaves, differing significantly from all other lines (Table 4). The same trends of individual leaf size among genetic lines were consistent within each soil type (Table 4) with one exception. In the Kansas soil, line 72, in addition to having significantly larger leaves compared to line 23 also had significantly larger leaves compared to control aspen (Table 4).

There was a significant effect of genetic line ($p<0.01$) on specific leaf area (SLA), but there was no effect of soil type and the genetic line × soil type

Table 3 Maximum photosynthetic rates (μmol CO_2 m^{-2} s^{-1}) of transgenic and control aspen (*Populus tremuloides*) grown in three soil types

	Line 72	Line 141	Line 23	Control	Line *p* values in each soil	Soil means
July						
Colorado	16.6 (0.9)^{a}	15.2 (0.6)^{ab}	16.7(0.6)^{a}	13.3 (0.3)^{b}	0.006	15.4 (0.4)^{a}
Kansas	16.4 (0.7)^{a}	15.0 (0.6)^{ab}	16.0 (0.7)^{a}	13.8 (0.3)^{bc}	0.062	15.4(0.4)^{a}
Texas	17.1 (1.1)	15.8 (0.9)	15.3 (1.5)	15.2 (0.8)	0.6	15.9 (0.5)^{a}
Line mean	16.7 (0.5)^{a}	15.3 (0.4)^{bc}	16 (0.5)^{ab}	14.1 (0.4)^{c}		
August						
Colorado	11 (0.8)^{a}	11.4 (0.7)^{ab}	12.9 (0.6)^{b}	10.1 (0.5)^{ac}	0.05	11.3 (0.4)^{a}
Kansas	13.5 (0.6)^{a}	11.9 (0.7)^{ab}	13 (1.2)^{a}	9.7 (0.7)^{bc}	0.07	12.3(0.5)^{a}
Texas	11.7 (1.3)	12.2 (0.4)	12.5 (0.8)	10.9 (0.5)	0.6	11.8 (0.4)^{a}
Line mean	12.1 (0.5)^{a}	11.8 (0.4)^{a}	12.8 (0.5)^{a}	10.3 (0.3)^{b}		
September						
Colorado	9.2 (0.9)	8.7 (1.0)	10.1 (0.9)	8.8 (1.6)	0.8	9.2 (0.5)^{a}
Kansas	9.6 (1.5)	10.6 (1.2)	8.3 (0.5)	11.5 (0.6)	0.2	10.0 (0.6)^{a}
Texas	10 (1.2)	11.1 (0.4)	9.9 (1.3)	11.3 (1.0)	0.7	10.6 (0.5)^{a}
Line mean	9.6 (0.6)	10.1 (0.7)	9.4 (0.5)	10.5 (0.7)		

For overall soil means, n=20; for overall genetic line means, n=15; for treatment means, n=5. p values displayed in the table were generated from one-way ANOVA examining the effect of genetic line within each soil type. Least significant difference post hoc results are displayed for $p<0.05$. Post hoc comparisons for genetic line are made across rows. Post hoc comparisons of soil means are made within columns for each measurement date.

interaction was not significant. Across soil types, the SLA for line 72 averaged 181±2 cm^2 g^{-1} and was significantly lower than the SLA of other lines of aspen (192±2 cm^2 g^{-1}), which did not differ significantly from each other.

Plant growth—accumulation and partitioning of carbon

Overall, there was a significant effect of soil type ($p<$ 0.001) and genetic line ($p<0.001$) on total plant C,

Table 4 Height (cm), plant total leaf area (cm^2), and individual leaf size (cm^2) of transgenic and control aspen (*Populus tremuloides*) grown in three soil types after 6 months of growth

	Line 72	Line 141	Line 23	Control	*p*	Soil means
Height (cm)						
Colorado	146^{a} (2.3)	189^{b} (1.9)	170^{c} (5.0)	182^{b} (3.4)	<0.001	172^{a} (3.3)
Kansas	149^{a} (2.6)	172^{b}(4.5)	162^{c} (1.8)	167^{bc} (1.8)	<0.001	162^{b} (2.1)
Texas	126^{a}(4.1)	157^{bc} (1.9)	151^{c} (3.0)	162^{b} (3.4)	<0.001	149^{c}(2.9)
Line means	140^{a} (2.7)	172^{b} (3.2)	161^{c} (2.5)	169^{b} (2.4)		
Total leaf area (cm^2)						
Colorado	3,149^{a} (135)	4,404^{b} (103)	3,530^{a} (240)	4,454^{b} (174)	<0.001	3,884^{a} (129)
Kansas	3,285^{a} (87)	4,096^{b} (79)	3,500^{a} (99)	3,888^{b} (96)	<0.001	3,692^{b} (71)
Texas	2,861^{a} (62)	3,376^{b} (119)	3,188^{b} (74)	3,843^{c} (115)	<0.001	3,317^{c} (78)
Line means	3,099^{a} (66)	3,959^{b} (106)	3,406^{c} (92)	4,062^{b} (93)		
Individual leaf size (cm^2per leaf)						
Colorado	62^{a} (1.2)	60^{a} (1.9)	52^{b} (2.7)	64^{a} (1.8)	0.001	60^{a} (1.2)
Kansas	61^{a} (1.0)	59^{ac} (1.1)	51^{b} (1.1)	56^{c} (1.73)	<0.001	57^{b} (0.9)
Texas	56^{a} (1.6)	50^{b} (1.2)	47^{b} (1.1)	56^{ca} (1.1)	<0.001	52^{c} (0.9)
Line means	60^{a} (0.2)	56^{b} (0.3)	50^{c} (0.2)	59^{a} (0.2)		

For overall soil means, n=32; for overall genetic line means, n=24; for treatment means, n=8. p values displayed in the table were generated from one-way ANOVA examining the effect of genetic line within each soil type. Least significant difference post hoc results are displayed for $p<0.05$. Post hoc comparisons for genetic line are made across rows. Post hoc comparisons of soil means are made within columns for each variable measured.

total stem C, total leaf C, and total aboveground C (stem C + leaf C), and the genetic line × soil type interaction was significant ($p<0.05$) Furthermore, there was a significant effect of soil type ($p<0.001$) and genetic line ($p<0.001$) on total root C; the interaction was not significant. Above to belowground biomass C ratios were significantly affected by genetic line ($p<0.001$), but were not affected by soil type; again the genetic line × soil type interaction was not significant.

Total plant C, total stem C, and total aboveground C of transgenic and control aspen differed significantly in all three soil types, with plants accumulating the most biomass C (total and components) in the Colorado soil and the least biomass C in the Texas soil (Fig. 1a). Aspen grown in Colorado soil accumulated significantly more total root C compared to the other two soil types, and aspen grown in Texas soil accumulated significantly less total leaf C compared to the other two soil types.

Across soils, line 72 accumulated 20% less total plant C, 20% less total stem C, 11% less total aboveground C, and 52% less total root C compared to all other genetic lines (Fig. 1b). Line 72 and line 23 had 10% less total leaf C compared to line 141 and control aspen, which did not differ significantly from each other (Fig. 1b). Line 72 also had the highest above to belowground C ratio (4.8) compared with ratios of other genetic lines (2.5; $p<0.001$), which did not differ significantly from each other.

Because of a significant genetic line by soil type interaction, we examined genetic line effects on biomass C within each soil type. The genetic line effects on total root, whole plant, and above/belowground C within soil types generally followed the trends from overall ANOVA (Fig. 1b; Table 5). Across soil types, line 72 accumulated significantly less stem C, leaf C, and total aboveground C than control aspen and similar patterns were observed for the Colorado and Texas soils. However, this was not the case in the Kansas soil, where there were no significant differences among genetic lines for aboveground biomass components (leaf C and aboveground C; Table 5).

Soil C formation

There were significant effects of soil type ($p<0.001$), genetic line ($p<0.001$), and a genetic line by soil type interaction ($p<0.01$) on soil C formation. Across genetic lines, we found the highest percentage of aspen-derived soil C in the Colorado soils, which were twice that of the Kansas and Texas soils ($p<0.001$). Aspen derived soil C in the Kansas and Texas soils did not differ significantly (Fig. 2a). Across soil types, line 72 accumulated 35% less new aspen-derived soil C compared to soil C formation under other genetic lines, which did not differ significantly from each other (Fig. 2b).

Because of the genetic line by soil type interaction, we examined differences in soil C formation among genetic lines in each soil type. For the Colorado soil, line 72 accumulated the lowest percentage of aspen-derived soil C and differed significantly from the other genetic lines (Fig. 3a). Line 141 and control aspen accumulated the highest percentages of aspen-derived soil C formation and differed from lines 23 and 72 (Fig. 3a). In the Kansas soil, there was no effect of genetic line on soil C formation (Fig. 3b). However, in the Texas soil, line 72 again accumulated the lowest percentage of aspen-derived soil C and differed significantly from all other genetic lines (Fig. 3c). Root biomass explained 48% and 29% of the variation in soil C formation in the Colorado and Texas soils (Fig. 4), respectively. There was not a significant linear relationship between root biomass and soil C formation in the Kansas soil (Fig. 4).

Discussion

Hypothesis 1: Effects of soil type and altered lignin biosynthesis on plant growth

Contrary to our first hypothesis (1a), soil type did significantly influence plant growth and biomass allocation, despite optimal fertilization and watering (Table 4, Fig. 1a). Though soils were selected primarily for their textural differences, they varied widely in several characteristics, including average pH, N content and C content (Table 1). Nonetheless, compared to other studies (Pastor and Post 1986; Silver et al. 2000; Anderson et al. 2006; Bouma and Bryla 2000), patterns were counter-intuitive. The Colorado soil had the highest sand content, the lowest clay content (Table 1) and was poorest in terms of extractable and total nutrients. However, plant growth rates were the highest in Colorado soils (Fig. 1a).

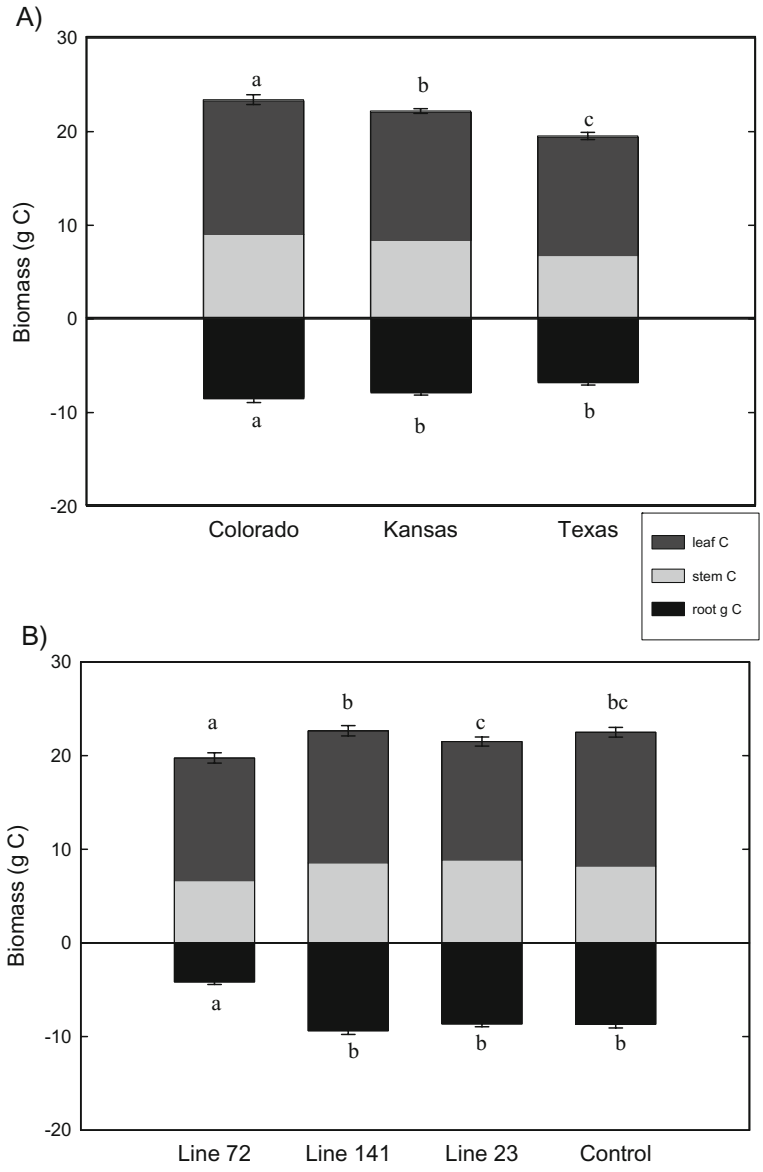

Fig. 1 Overall distribution of total carbon (g C) in aboveground and belowground pools in **a** three different soils types and **b** transgenic and control aspen after 6 months of greenhouse growth. Least significant difference post hoc results displayed for total aboveground C and root C are significant at $p<0.05$

Perhaps the negative surface charge of clays and the formation of aggregates in high clay content soils retained nutrients making them inaccessible to plant roots (Brady and Weil 2002). Additionally, nutrient availability, especially phosphorus is highest in a pH range of 6.0 to 7.0. At pH values higher than this, phosphorous is fixed into calcium phosphates and tends to be unavailable to plants (Brady and Weil 2002). The availability of iron also declines with increasing pH. The Colorado soil had a pH of 6.4, while the Kansas and Texas soils had pH values of 7.7 and 7.9, respectively (Table 1). Finally, aspen are known to be sensitive to poorly drained soils (Graham et al. 1963; Gustafson et al. 2003). The Texas soil had 40% more clay than the Kansas soil and 69% more clay than the Colorado soil (Table 1) and drained more slowly after watering (personal observations, J.H. and K.B.), perhaps resulting in periods of sub-optimal (high) soil moisture.

Based on our previous results, we anticipated that aboveground biomass would not be affected in low lignin lines, but that root biomass would be reduced (hypothesis 1b). While low lignin aspen (lines 23 and 141) accumulated similar amounts of total aboveground C compared to control aspen, root biomass

Table 5 Distribution of total carbon (g C) in aboveground and belowground pools in transgenic and control aspen (*Populus tremuloides*) after 6 months of growth

	Line 72	Line 141	Line 23	Control
Root				
Colorado	4.4 (0.46)^{a}	10.8 (0.83)^{b}	9.4 (0.70)^{b}	9.9 (0.67)^{b}
Kansas	4.7 (0.14)^{a}	9.3 (0.41)^{b}	8.9 (0.4)^{b}	8.7 (0.70)^{b}
Texas	3.4 (0.67)^{a}	8.3 (0.41)^{b}	7.7 (0.22)^{b}	7.5 (0.48)^{b}
Stem				
Colorado	7.2 (0.35)^{a}	10.0 (0.23)^{b}	9.4 (0.62)^{b}	9.6 (0.40)^{b}
Kansas	7.9 (0.32)^{ab}	8.5 (0.25)^{a}	9.2 (0.17)^{c}	7.9 (0.15)^{b}
Texas	4.8 (0.38)^{a}	7.1 (0.22)^{b}	8.0 (0.21)^{c}	7.2 (0.27)^{b}
Leaf				
Colorado	13.5 (0.31)^{a}	15.4 (0.59)^{b}	12.9 (0.68)^{a}	15.2 (0.56)^{b}
Kansas	14.0 (0.29)^{a}	14.1 (0.26)^{a}	12.9 (0.17)^{a}	13.7 (0.57)^{a}
Texas	11.8 (0.17)^{a}	12.8 (0.36)^{b}	12.1 (0.25)^{ab}	13.9 (0.34)^{c}
Total aboveground				
Colorado	20.7 (0.56)^{a}	25.3 (0.52)^{b}	22.3 (1.26)^{a}	24.8 (0.93)^{b}
Kansas	21.9 (0.56)^{a}	22.7 (0.50)^{a}	22.1 (0.31)^{a}	21.6 (0.60)^{a}
Texas	16.6 (0.36)^{a}	19.9 (0.50)^{b}	20.1 (0.44)^{b}	21.1 (0.55)^{b}
Total plant				
Colorado	25.1 (0.85)^{a}	35.7 (0.86)^{b}	31.7 (1.72)^{c}	34.7 (1.55)^{bc}
Kansas	26.6 (0.57)^{a}	32.0 (0.53)^{b}	31.0 (0.60)^{b}	30.3 (0.91)^{b}
Texas	20.2 (1.00)^{a}	28.1 (0.80)^{b}	27.8 (0.50)^{b}	28.6 (0.89)^{b}
Aboveground to belowground ratio				
Colorado	5.1 (0.58)^{a}	2.4 (0.22)^{b}	2.4 (0.13)^{b}	2.6 (0.12)^{b}
Kansas	4.7 (0.20)^{a}	2.5 (0.12)^{b}	2.5 (0.10)^{b}	2.6 (0.32)^{b}
Texas	5.6 (0.55)^{a}	2.4 (0.11)^{b}	2.6 (0.09)^{b}	2.9 (0.17)^{b}

Values are treatment means ± SE (n=8). p values for values in the table were generated from one-way ANOVA examining the effect of genetic line within each soil type. Least significant difference post hoc results are displayed across rows for $p<0.05$.

did not differ between control aspen and low lignin lines (Fig. 1b). A goal of our second experiment was to assess the trait stability of the lignin modified plants, and our results suggest that growth characteristics may vary across trials and so may depend on or be influenced by several factors other than the genetic modification. Subtle variation in micro-propagation techniques or propagate handling between experiments or inter-annual variation in greenhouse climate may have affected plant performance. There was variation in the growth and biomass allocation patterns of individual lines in our previous study, and it could be that modified aspen are somewhat plastic in their response to subtle changes in environmental conditions. For example, out-planting in the current experiment occurred 30 days later than our first experiment and the current experiment was 30 days shorter (harvest occurred on similar dates). To the fullest extent possible, we attempted to control for all other aspects of the two experiments: both relied on the same greenhouse facility for the experiments, the same water and nutrient amendment regimes, and for the Kansas soils, a sampling area <20 m from the sampling area used for the previous experiment.

We hypothesized, based on previous results (Hancock et al. 2007), that high S/G aspen (line 72) would have reduced aboveground and belowground biomass (hypothesis 1b). Our results supported this hypothesis (Table 4, Fig. 1b). As discussed in Hancock et al. (2007), the C cost of syringyl production is higher compared to guaiacyl production (Amthor 2003). However, this increased cost of higher S/G cannot fully explain the large reductions in growth and alterations to biomass accumulation in high S/G aspen,

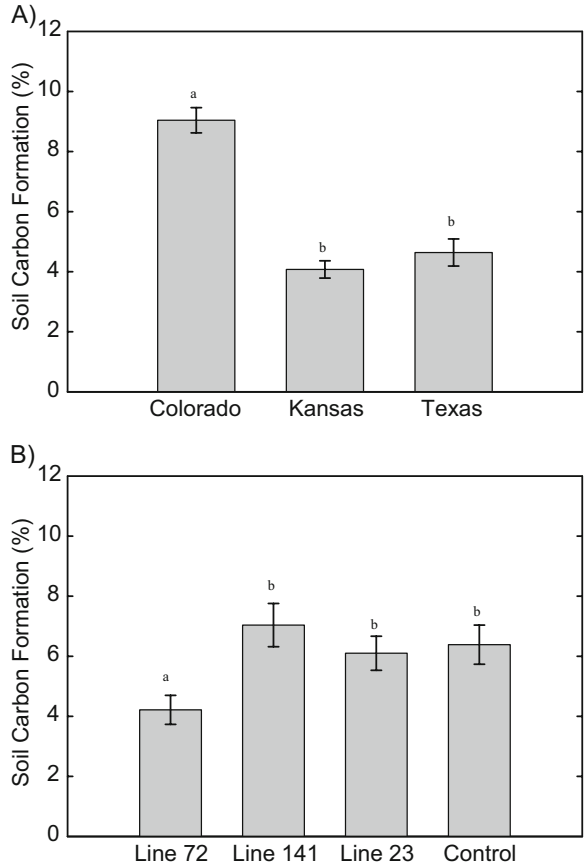

Fig. 2 Overall estimates of percent new soil carbon (C) derived from isotope mixing models **a** among three different soils types and **b** compared among genetic lines averaged across soil types. Post hoc (least significant difference) results are significant at $p<0.05$

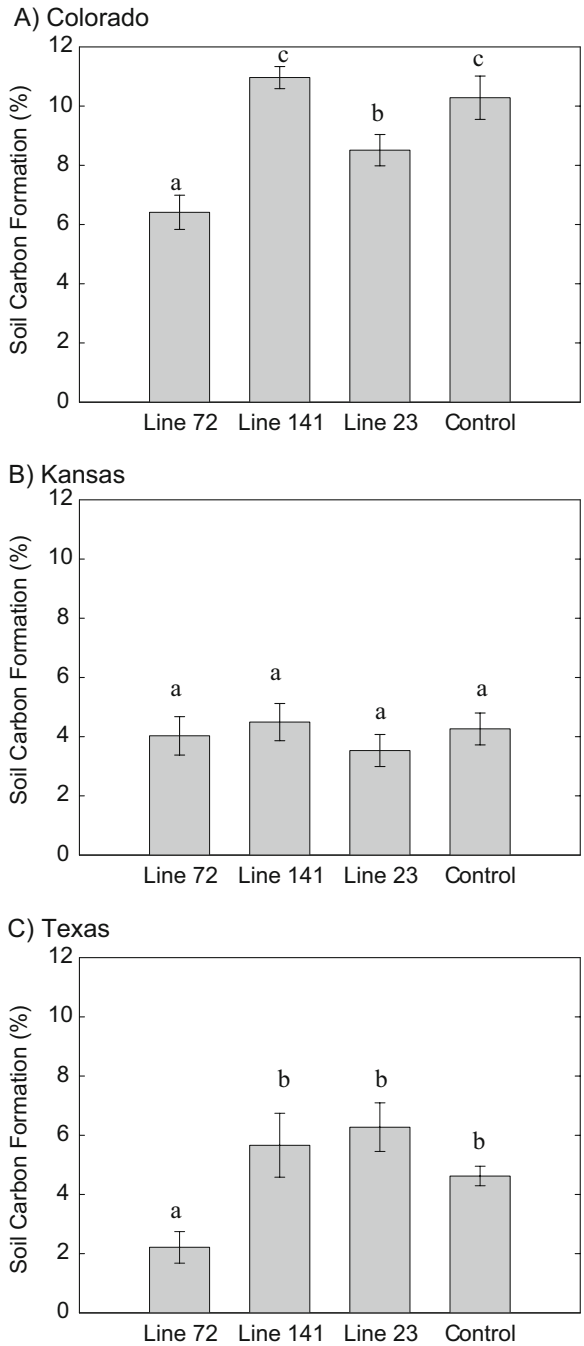

Fig. 3 Estimates of percent new soil carbon (C) derived from isotope mixing models of transgenic and control aspen grown for 6 months in **a** Colorado soil, **b** Kansas soil, and **c** Texas soil. Post hoc (least significant difference) results are significant at $p<0.05$

and likely relates to manipulations of S/G biosynthesis having unintended consequences for plant growth and biomass allocation (Hancock et al. 2007).

Genetic line effects on above and belowground biomass allocation were generally consistent within the Colorado and Texas soil types (Table 5). In particular, aboveground C of line 72 was significantly reduced in the Texas and Colorado soils, but there were no differences in aboveground C among genetic lines in the Kansas soil. This contrasting trend in the Kansas soil could be attributed to larger individual leaf size of line 72 when compared to control aspen (Table 4). Larger leaves of line 72 combined with higher photosynthetic rates in the Kansas soil could have led to increased C growth and led to a lack of differences among genetic lines in this soil.

Hypothesis 2: Effects of soil type and genetic line on soil carbon formation

We hypothesized that plant growth would not differ in the three soil types used in this experiment, and that detrital inputs to soil would be similar among soil types. Therefore, we predicted that soil properties would control soil C formation in this study and that the Colorado soil, which had the lowest percent clay content and highest percent sand content (Table 1), would have the lowest rates of soil C formation (Six et al. 2002), while the Texas soil, which had the highest percent clay content, would have the highest rates of soil C formation (hypothesis 2). Instead, we observed that the Colorado soil had a significantly higher percentage of new aspen-derived soil C compared to the Kansas and Texas soils, which did not differ significantly from each other (Fig. 2a).

The differences we found in soil C formation among soil types can be attributed to observed differences in plant biomass and allocation across soil types, differences in clay content, and to differences in microbial biomass (Fig. 1b). Using a method adapted from Frostegård et al. (1991), we estimated soil microbial biomass in each soil type using total phospholipid fatty acid (PLFA) analysis (Bradley et al. 2007). The Colorado soil had the least total microbial biomass (PLFA=48±2.5 nm C g^{-1} soil), the Texas soil had intermediate values of microbial biomass (PLFA=131±4.8 nmol C g^{-1} soil), and the Kansas soil had the highest microbial biomass (PLFA=160±4.1 nmol C g^{-1} soil; Bradley et al. 2007). Thus, although we expected higher decomposition rates in the Colorado soil due to ideal physical conditions (aeration, moisture), the high percentage of new

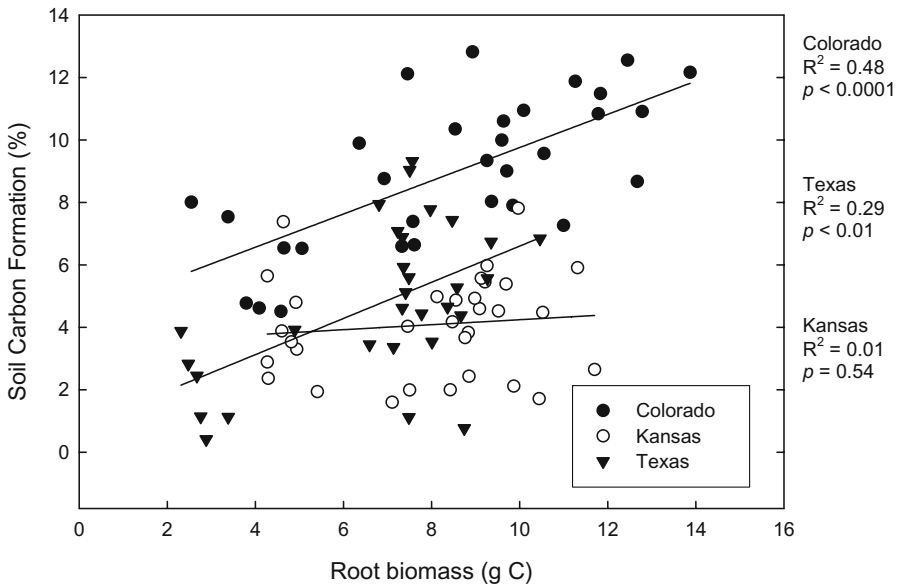

Fig. 4 Soil carbon (C) formation as a function of root biomass (g C) in each soil type. Post hoc (least significant difference) results are significant at $p<0.05$

aspen-derived C in the Colorado soil can most likely be attributed to lower heterotrophic decomposition and respiration, combined with greater C root inputs relative to the other two soil types (Fig. 1a). This is supported by findings of Zak at al. (1994), which showed higher incubation-based respiration rates at similar Kansas sites than similar Colorado sites. Kansas had high silt and clay content (Table 1) and intermediate C inputs (Fig. 1a). However, high microbial biomass in these soils (Bradley et al. 2007) most likely led to high rates of heterotrophic decomposition of C inputs and thus to the lowest rates of soil C formation of the three soil types. Finally, while Texas soil had the lowest belowground root mass (Fig. 1a), these soils supported intermediate values of microbial biomass (Bradley et al. 2007), which when combined with the physical protection of aggregates in the Texas soil may have led to less microbial decomposition of C inputs and slightly higher (but not significantly so) soil C formation rates (Fig. 2a).

In our previous study (Hancock et al. 2007), soil C formation was significantly related to root biomass, with large reductions in root biomass in high S/G aspen resulting in 70% less new aspen derived soil C compared to the control lines. Additionally, 15–17% reductions in root biomass in low lignin lines contributed to 33–43% less new soil C (Hancock et al. 2007). In the current experiment, we also found that aspen with a 60% increase in S/G (line 72) accumulated 35% less new soil C compared to control aspen (Fig. 2b). However, we did not observe differences in belowground biomass in lines 23 and 141. New soil C in these low lignin lines also did not differ from control aspen (Fig. 2b). Overall, it appears that rates of new soil C formation were related to differences in root biomass, but reductions in root biomass were not consistent across experiments despite utilizing the same low lignin lines (23, 141). Again, subtle differences across experiments with respect to micropropagation, study length, greenhouse environment could explain these differences across studies.

Genetic line effects were generally consistent in the Colorado and Texas soils (Fig. 3a and c) with line 72 having significantly less new soil C compared to other lines of aspen. However, in the Kansas soil, there was no effect of genetic line on soil C formation (Fig. 3b). Growth performance and soil C formation of the high S/G line used in this experiment differed from the high S/G line used in our previous experiment (Hancock et al. 2007) in which aspen were also grown in the Kansas soil. In contrast to the high S/G line used in this experiment, which had a 60% increase in S/G accompanied with a 40% decrease in lignin content, the high S/G lignin line (93) used in our previous experiment had a 150% increase in S/G with no modifications to lignin content. Therefore, the less dramatic changes in lignin quantity combined with a smaller increase in S/G in line 72 appear to have resulted in better growth in Kansas soil

compared to the high S/G line used previously (Hancock et al. 2007; line 93).

Despite reduced belowground inputs in the Kansas soil, line 72 had larger individual leaf size (Table 4), higher photosynthetic rates (Table 3), and did not have reduced aboveground biomass compared to other lines in the Kansas soil (Table 5). Given these characteristics, this high S/G line may have been able to allocate more photosynthate belowground to root exudates (Kuzyakov and Domanski 2000; Thornton et al. 2004) resulting in similar rates of soil C formation compared to other lines of aspen in the Kansas soil. Overall, however, variation in root biomass was not related to rates of soil C formation in the Kansas soil (Fig. 4), suggesting that the lack of difference between the four lines may have resulted for other reasons. For example, Bradley et al. (2007) showed that the activities of two enzymes responsible for the degradation of cellulose (cellubiohydrolase and β-glucosidase) were lower in lines 72 and 141 than in the control line in the Kansas soil. Further the abundance of fungal biomarkers (neutral lipid fatty acids) associated with decomposers were also reduced in line 72. These reductions in turn would lower C decomposition rates in line 72 and141 compared to the other lines. Overall, the reduction in the abundance of organisms and enzymes associated with decomposition in the Kansas soil may have offset lower root inputs and resulted in similar rates of C formation across the four lines. Unfortunately, we do not have similar analyses for the original experiment.

Conclusions

We found a strong relationship between root biomass and soil C formation across two of the three soil types. As with our earlier study, modified aspen expressing increased S/G lignin (line 72) had significantly reduced height, leaf area, and total biomass as well as reductions in aspen-derived soil C. In contrast to our previous experiment, there were no negative impacts of reduced lignin biosynthesis (lines 23 and 141) on plant growth or soil C formation suggesting that expression of traits is highly sensitive to subtle differences in propagation techniques or environment. Alternatively, expression of traits may not be stable over time. Future efforts should work towards a more complete understanding of the stability of genetic transformation through micropropagation and plant establishment.

Our work also demonstrates that transgenic aspen performance and subsequent effects on soil C formation are affected by soil type, and long-term field studies across a broad range of soil types are necessary before genetically modified trees are deployed for commercial purposes. Overall, because the wild type line was generally the best performer across soils, and there is tremendous genetic variation within aspen, it would be valuable to examine diverse naturally occurring lines of aspen for both tissue quality and growth characteristics. It may be possible to identify aspen lines that naturally express both reduced lignin properties but higher growth rates. Although altering lignin biosynthesis in aspen could be a potential tool for reducing the economic and environmental costs of Kraft pulping, particularly the high S/G aspen lines, our limited studies suggest that genetic modification of aspen may lower growth performance compared to wild-type aspen.

Acknowledgements The authors would like to thank Cassie Miller and Noah Karberg for their assistance in the greenhouse and the lab and Drs. Chung-Jui and Scott Harding for invaluable advice on plant maintenance. Additionally we would like to thank Dr. John Adler and the Department of Biological Sciences at Michigan Technological University for use of greenhouse facilities. We also thank the Pawnee National Grassland, Konza Prairie Biological Station, and the Blackland Research and Extension Center in Temple Texas, for access to soils used in this experiment. Finally, we greatly appreciate funding and support from the US Department of Energy, the Northern and Pacific Southwest Research Stations of the USDA Forest Service, and the graduate school of Michigan Technological University.

References

Amthor JS (2003) Efficiency of lignin biosynthesis: a quantitative analysis. Ann Botony 9:673–695

Anderson TM, Dong Y, McNaughton SJ (2006) Nutrient acquisition and physiological responses of dominant Serengeti grasses to variation in soil texture and grazing. J Ecol 94:1164–1175

Atjay G, Ketner P, Duvigneaud D (1979) Terrestrial primary production and phytomass. In: Bolin B, Degens E, Kempe S, Ketner P (eds) The global C cycle. Wiley, Chichester, UK, pp 129–181

Barnes BV, Wagner WH (2004) Michigan trees. University of Michigan Press, Ann Arbor, p 447

Baucher M, Chabbert B, Pilate G, Van Doorsselaere J, Tollier M-T, Petit-Conil M, Cornu D, Monties B, Van Montagu M, Inzé D, Jouanin L, Boerjan W (1996) Red xylem and

higher lignin extraction extractability by down regulating a cinnamyl alcohol dehydrogenase in poplar. Plant Physiol 112:1479–1490

Baucher M, Halpin C, Petit-Conil M, Boerjan W (2003) Lignin: Genetic engineering and impact on pulping. Crit Rev Biochem Mol Biol 38:305–350

Berg B, Lundmark J-E (1985) Decomposition of needles and root litter in lodgepole pine and Scots pine monocultural systems. Plant Soil 138:123–132

Bonde T, Christensen BT, Cerri CC (1992) Dynamics of organic matter as reflected by natural ^{13}C abundance in the particle size fractions of forested and cultivated oxisols. Soil Biol Biochem 24:275–277

Bouma TJ, Bryla DR (2000) On the assessment of root and soil respiration for soils of different textures: interactions with soil moisture contents and soil CO_2 concentrations. Plant Soil 227:215–221

Bradley KL, Hancock JE, Giardina CP, Pregitzer KS (2007) Soil microbial community responses to altered lignin biosynthesis in *Populus tremuloides* vary among three distinct soils. Plant Soil 294:185–201

Bradshaw HD, Ceulemans R, Davis J, Stettler R (2000) Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. J Plant Growth Regul 19: 306–313

Brady NC, Weil RR (2002) The nature and properties of soils. Prentice Hall, Upper Saddle River, p 960

Brunner AM, Busov VB, Strauss SH (2004) Poplar genome sequence: functional genomics in an ecologically dominant plant species. Trends Plant Sci 9:49–56

Chen C, Baucher M, Christensen JH, Boerjan W (2001) Biotechnology in trees: towards improved paper pulping by lignin engineer. Euphytica 118:185–195

Christensen BT (1987) Decomposability of organic matter in particle size fractions from field soils with straw incorporation. Soil Biol Biochem 19:429–435

Cox MS, Gerard PD, Abshire MJ (2006) Selected soil properties' variability and their relationships with yield in three Mississippi fields. Soil Sci 171:541–551

Desjardins T, Andreux F, Volkoff B, Cerri CC (1994) Organic carbon and ^{13}C contents in soils and soil size fractions, and their changes due to deforestation and pasture installation in eastern Amazonia. Geoderma 61:103–108

Fisher RF, Binkley D (2000) Ecology and management of forest soils. Wiley, New York, p 489

Frostegård Å, Tunlid A, Bååth E (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. J Microbiol Meth 14:151–163

Gale WJ, Cambardella CA, Bailey TB (2000) Root-derived C and the formation and stabilization of aggregates. Soil Sci Soc Am J 64:201–207

Giardina CP, Ryan MG, Hubbard RM, Binkley D (2001) Tree species and soil textural controls on carbon and nitrogen mineralization rates. Soil Sci Soc Am J 65:1272–1279

Graham SA, Harrison RP, Westell CE (1963) Aspens: Phoenix trees of the great lakes region. University of Michigan Press, Ann Arbor, p 272

Gustafson EJ, Lietz SM, Wright JL (2003) Predicting the spatial distribution of aspen growth potential in the upper great lakes region. For Scie 49:499–508

Halpin C, Thain SC, Tilston EL, Guiney E, Lapierre C, Hopkins DW (2007) Ecological impacts of trees with modified lignin. Tree Genet Genomes 3:101–110

Hancock JE, Loya WM, Giardina CP, Li L, Chiang VL, Pregitzer KS (2007) Plant growth, biomass partitioning and soil carbon formation in response to altered lignin biosynthesis in *Populus tremuloides*. New Phytol 173:732–742

Harding SA, Leshkevich J, Chiang VL, Tsai C-J (2002) Differential substrate inhibition couples kinetically distinct 4-coumarate:conenzyme A ligases with spatially distinct metabolic roles in quaking aspen. Plant Physiol 128:428–438

Hassink J (1997) The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant Soil 191:77–87

Herrera S (2005) Struggling to see the forest through the trees. Nature Biotechnol 23:165–167

Ho CK, Chang S-H, Tsay J-Y, Tsai C-J, Chiang VL, Chen Z-Z (1998) Agrobacterium tumefaciens-mediated transformation in Eucalyptus camaldulensis and production of transgenic plants. Plant Cell Rep 17:675–680

Hobbie SE, Reich PB, Oleksyn J, Ogdahl M, Zytkowiak R, Hale C, Karolewski P (2006) Tree species effects on decomposition and forest floor dynamics in a common garden. Ecology 87:2288–2297

Hu W-J, Harding SA, Lung J, Popko JL, Ralph J, Stokke DD, Tsai C-J, Chiang VL (1999) Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. Nature Biotechnol 17:808–812

Jenkinson DS (1988) Soil organic matter and its dynamics. In: Wild A (ed) Russel's soil conditions and plant growth. Longman, New York, pp 564–607

Jobbagy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl 10:423–436

Kuzyakov Y, Domanski G (2000) Carbon inputs by plants into the soil. Rev J Plant Nutr Soil Sci 163:421–431

Li L, Zhou Y, Cheng X, Sun J, Marita JM, Ralph J, Chiang VL (2003) Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. Proc Natl Acad Sci USA 100:4939–4944

Lorenz K, Lal R (2005) The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. Adv Agron 88:35–66

Meentemeyer V (1978) Macroclimate and lignin control of litter decomposition rates. Ecology 59:465–472

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626

Parton W, Silver WL, Burke IC, Grassens L, Harmon ME, Currie WS, King JY, Adair EC, Brandt LA, Hart SC, Fasth B (2007) Global-scale similarities in nitrogen release patterns during long term decomposition. Science 315: 361–364

Pastor J, Post WM (1986) Influence of climate, soil moisture, and succession on forest carbon and nitrogen cycles. Biogeochemistry 2:3–27

Pilate G, Guiney E, Holt K, Petit-Conil M, Lapierre C, Leple JC, Pollet B, Mila I, Webster EA, Marstorp HG, Hopkins

DW, Jouanin L, Boerjan W, Schuch W, Cornu D, Halpin C (2002) Field and pulping performances of transgenic trees with altered lignification. Nat Biotechnol 20:607–612

Russell AE, Raich JW, Valverde-Barrantes OJ, Fisher RF (2007) Tree species effects on soil properties in experimental plantations in tropical moist forest. Soil Sci Soc Amer J 71:1389–1397

Schimel DS, Braswell BH, Holland EA, Mckeown R, Ojima DS, Painter TH, Parton WJ, Townsend AR (1994) Climatic, edaphic, and biotic controls over storage and turnover of C in soils. Glob Biogeochem Cycles 8:279–293

Silver WL, Neff J, McGroddy M, Veldkamp E, Keller M, Cosme R (2000) Effects of soil texture on belowground carbon and nutrient storage in a lowland Amazonian forest ecosystem. Ecosystems 3:193–209

Six J, Conant RT, Paul A, Paustian K (2002) Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241:155–176

Sorensen LH (1972) Stabilization of newly formed amino acid metabolites in soil by clay minerals. Soil Sci 114:5–11

Stevenson FJ (1994) Humus Chemistry: genesis, composition, reactions. Wiley, New York, p 512

Strauss SH, DiFazio SP, Meilan R (2001) Genetically modified poplars in context. For Chron 77:271–279

Thornton B, Paterson E, Midwood A, Sim A, Pratt SM (2004) Contribution of current carbon assimilation in supplying root exudates of *Lolium perenne* measured using steady-state ^{13}C labeling. Physiol Plant 120:434–441

Tilston EL, Halpin C, Hopkins DW (2004) Genetic modifications to lignin biosynthesis in field-grown poplar trees have inconsistent effects on the rate of woody trunk decomposition. Soil Biol Biochem 36:1903–1906

Torn MS, Trumbore SE, Vitousek PM (1997) Mineral control of soil organic carbon storage and turnover. Nature 389:170–173

Tsai C-J, Podila GK, Chiang VL (1994) *Agrobacterium*-mediated transformation of quaking aspen (*Populus tremuloides*) and regeneration of transgenic plants. Plant Cell Rep 14:64–97

Tsai C-J, Popko JL, Miclke MR, Hu W-J, Podila GK, Chiang VL (1998) Suppression of *O*-methyltransferase gene by homologous sense transgene in quaking aspen causes red-brown wood pheonotypes. Plant Physiol 117:101–112

Van Veen JA, Kuikman PJ (1990) Soil Structural aspects of decomposition of organic matter by microorganisms. Biogeochem 11:213–233

Zak DR, Tilman D, Parmenter RR, Rice CW, Fisher FM, Vose J, Michunas D, Martin CW (1994) Plant production and soil microorganisms in late successional ecosystems: a continental-scale study. Ecology 75:2333–2347