

**GREENHOUSE GAS FLUXES AND ROOT PRODUCTIVITY IN A SWITCHGRASS
AND LOBLOLLY PINE INTERCROPPING SYSTEM FOR BIOENERGY
PRODUCTION**

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GREENHOUSE GAS FLUXES AND ROOT PRODUCTIVITY IN A SWITCHGRASS AND LOBLOLLY PINE INTERCROPPING SYSTEM FOR BIOENERGY PRODUCTION

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(ABSTRACT)

This study is part of a larger collaborative effort to determine the overall environmental sustainability of intercropping pine (*Pinus taeda* L.) and switchgrass (*Panicum virgatum* L.), both of which are promising feedstock for bioenergy production in the Lower Coastal Plain in North Carolina. We measured soil CO₂ efflux (R_S) every six weeks from January 2012 to March 2013 in four-year-old monoculture and intercropped stands of loblolly pine and switchgrass. R_S is primarily the result of root respiration (R_A) and microbial decomposition of organic matter (R_H) releasing CO₂ as a by-product and is an important and large part of the global carbon (C) cycle. Accurate estimates of the two components of total soil respiration (R_S) are required as they are functionally different processes and vary greatly spatially and temporally with species composition, temperature, moisture, productivity, and management activities. We quantified R_A and R_H components of R_S by using a root exclusion core technique based on root carbohydrate depletion, which eliminates R_A within the cores over time. We determined the relationship between R_S, R_A and R_H measurements and roots collected from the cores. We took fresh soil cores in July 2012 to compare root productivity of loblolly pine and switchgrass in monoculture versus the co-culture. Additionally, CH₄ and N₂O fluxes were monitored quarterly using vented static chambers. Pure switchgrass had significantly higher R_S rates (July, August, September), root biomass and root length in the top 0-35 cm relative to switchgrass in the co-culture, while loblolly pine with and without switchgrass had no significant changes in R_S and roots. Correlations between R_A and roots showed significantly positive correlation of R_A to grass root biomass ($r = 0.37$, $p \leq 0.001$), fine ($r = 0.26$, $p \leq 0.05$) and medium root surface area ($r = 0.20$, $p \leq 0.1$). The estimated portions of R_S attributed to R_A in the intercrop stand were 31% and 22% in the summer and fall, respectively. No significant treatment differences were observed in either CH₄ or N₂O flux. Our study indicates a decrease in switchgrass root productivity in the intercropped stand versus the monoculture stand which could account for differences in the observed R_S.

Dedication

I would like to dedicate this work to my parents, Gayatri Shrestha and Bholu Shrestha for making it possible for me to participate in such a project in the first place, my brother Binayak, and my beloved Nelish for his endless love and support.

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INTRODUCTION & JUSTIFICATION

Research Rationale

The burning of fossil fuels and its associated threats to public health and environmental sustainability are of great concern. Economic and national security concerns as a result of overdependence on fossil fuels have led to creating avenues for developing alternative sources of renewable energy which include bioenergy and biofuels. The U.S. Department of Energy (DOE), partnering with U.S. Department of Agriculture (USDA), has selected switchgrass (*Panicum virgatum*) as the model feedstock to be used for biofuel production, particularly in the Southeastern U.S. (Ma et al. 2000) where the favorable climate of the region could lead to higher productivity of this crop. DOE believes that biofuels made from high yielding native perennial forage crops such as switchgrass could reduce the reliance on foreign oil supplies, curb emissions of greenhouse gases (mainly CO₂), and strengthen rural farm economy (McLaughlin et al. 1999). Biofuels are liquid fuels made out of plant sugars (from crops like sugarcane and corn) and cellulosic materials (from wood by-products and high fiber grasses like switchgrass) by fermentation to make ethanol, a transportation fuel (McLaughlin et al 1999, ATTRA 2006).

Switchgrass and Loblolly Pine

The loblolly pine and switchgrass intercropping system for biofuel production is a unique endeavor and has not been widely studied. Loblolly pine is a native tree species to the U.S. with a range that extends from New Jersey to Florida and west to eastern Texas. It is the most commercially important pine species of southeastern U.S. Intensively managed plantations in the southeastern U.S. rely heavily on loblolly pines (Munsell and Fox 2010) because the trees

reproduce and grow rapidly in diverse conditions resulting in shorter rotation periods for landowners. Loblolly pines have a large per hectare yield and provide versatile marketable products, making it the most favorable pine species in the south (Schultz 1997).

Switchgrass is a fast growing herbaceous crop native to Central and North American tall grass prairie, and can accumulate high biomass, both below and aboveground. It has low nutrient requirements and high nitrogen (N) use and water use efficiency (McLaughlin et al. 1999). While it can reach up to 10 meters in height, its roots extend vertically down to more than 3.5 meters (McLaughlin et al. 1999) enabling the plant to successfully acquire resources even under stressful conditions. Two major ecotypes of switchgrass exist, an upland type (thinner stemmed) that favors drier soils and semi-arid climates of the northern U.S., and lowland varieties (thicker stemmed) adapted to warmer and moister conditions in the southern range.

Intercropping switchgrass and loblolly pine

Intercropping switchgrass into traditional loblolly pine plantations can provide several benefits to this managed forest ecosystem. Annual harvests of switchgrass at the end of each growing season provide valuable feedstock for biofuel production. Switchgrass, by exploiting additional soil volume through its long root systems, can contribute to sequestration of soil carbon (C) providing valuable ecosystem service. Switchgrass also has high N use efficiency (McLaughlin et al. 1999) and can prevent mineralized N losses, particularly leaching of mobile nutrients such as nitrate from the system via uptake. Further, utilizing the available space under the pine plantation for switchgrass can help to control competing, less valuable woody species (e.g., sweetgum) including volunteer loblolly pine.

Importance of soil CO₂ efflux and trace gas exchange

Soils are of special importance in climate science as they store the largest amount of C and N found in terrestrial ecosystems (Bowden et al. 2004, Schlesinger 1997 respectively), mainly in the form of organic matter. Globally, soils contain twice as much C (1576 Pg) as the atmosphere (760 Pg; Eswaran et al. 1993), although, terrestrial vegetation stores approximately 560 Pg C, while the oceans contain around 38,000 Pg C and are the largest reservoir of the global C pool (Rustad et al. 2000, Schlesinger and Andrews 2000). The 1500 Pg C estimate for soils is based on data where soil organic C was measured only on the top meter of the soil (Eswaran et al. 1993). Recent studies of soil organic C estimate an additional 850 Pg C existing in the second and third meter of soil globally (Jobbagy and Jackson 2000).

Microbial decomposition of soil organic matter (SOM) and root respiration are two major processes by which stored C from forest ecosystems is released into the atmosphere as CO₂ (Raich and Schlesinger 1992). A majority of the C inputs to forest soils are driven by photosynthesis, which subsequently gives rise to root matter, litter inputs and root exudates that make their way into organic and mineral soil (Schlesinger 1977).

Although CO₂ is considered to be the most important greenhouse gas being produced in largest quantities, methane (CH₄) and nitrous oxide (N₂O), which are emitted in smaller quantities, also substantially contribute to global warming (Smith et al. 2003). Greenhouse gases absorb long wave radiation emitted from the earth's surface thereby contributing to warming. Both CH₄ and N₂O are radiatively active and potent greenhouse gases having greater warming potential than CO₂ in the atmosphere. The warming potential of 1 kg of CH₄ is 23 times greater than that of CO₂, while that of N₂O is 300 times greater (Smith et al. 2003). Nearly a third of

CH₄ emissions and two-thirds of N₂O emissions to the atmosphere come from soil (Smith et al. 2003).

This research is part of a larger effort to study the benefits of developing loblolly pine (*Pinus taeda* L.) and switchgrass (*Panicum virgatu* L.) intercropping systems for bioenergy production in the southeastern United States. The broad goal of the project is to determine the economic and environmental sustainability of these systems. My research will specifically focus on the effects of intercropping loblolly pine and switchgrass on soil CO₂ efflux, or soil respiration (R_S), and CH₄ and N₂O trace gas fluxes at the soil atmosphere interface. R_S is defined as the CO₂ released from respiration of soil heterotrophs (e.g., microbes and soil fauna which decompose organic substrates) and roots and root-associated mycorrhizal fungi (Raich and Schlesinger 1992, Boone et al. 1998, Lamberty et al. 2011). Increased storage of C in the soil could help offset the effects of anthropogenic emission of CO₂ and improve soil physical and chemical properties and overall sustainability of the system (Rustad et al. 2000). We will further investigate the separation of R_S into its autotrophic (R_A; root and mycorrhizal) and heterotrophic (R_H; microbial) components to understand how the spatial variation in the two components in this system drive R_S. R_A and R_H vary greatly spatially with species composition, temperature, moisture, NPP, litter, soil type and management activities (Bond-Lamberty et al. 2011, Guay et al. 2007). The relative contribution of R_A and R_H also varies with the time of the year with the contribution from R_A to R_S increasing in summer as photosynthesis along with root production is at its peak (Guay et al. 2007).

Specific Objectives:

1. Compare R_S in pine, switchgrass, and pine + switchgrass intercropped production systems.
2. Compare the relative contribution of R_A and R_H to R_S from pine + switchgrass intercropped treatment.
3. Quantify trace gas fluxes of CH_4 and N_2O from pine and pine + switchgrass intercropped treatments.
4. Examine how belowground root distribution and root productivity of loblolly pine and switchgrass varies among pine, switchgrass, and pine + switchgrass treatments.
5. Examine the relationship of root distribution to R_S , R_A , and R_H .

LITERATURE REVIEW

Soil Respiration

Total R_S can be used to calculate the amount of C released annually from a system (Fenn et al. 2010). Therefore, quantifying R_S from intensively managed forest ecosystems can be important as it allows us to develop forest C budgets and understand the role of managed forests in C sequestration (Pangle and Seiler 2002). In fact, the amount of CO_2 released from R_S makes up a majority (e.g., over two-thirds) of the total ecosystem respiration in temperate forests (Davidson et al. 1998, Gaumont-Guay et al. 2007). Carbon sequestration in forest ecosystems is a result of the difference between C uptake by plants through photosynthesis and ecosystem respiratory losses of C from all plant parts and soils. Any changes in R_S will influence net ecosystem productivity (NEP) and thus the potential of a system to sequester C (Kelting et al. 1998).

Behind photosynthesis, R_S is the second largest terrestrial flux of C in the global C cycle (Schlesinger and Andrews 2000) producing 75-80 Pg of CO_2 -C annually (Raich et al. 2002). Soil respiration has the potential role to accelerate increases in atmospheric CO_2 concentration (Tang et al. 2003). Small changes in the soil C pool via changes in soil respiration rates, in which CO_2 is released, can alter rates of soil C sequestration (Bowden et al. 2004).

R_S is also used as a parameter to measure total soil biological activity including rates of microbial activity, root dynamics and nutrient turnover (Raich and Schlesinger 1992), processes closely related to sustainability of silvicultural systems. Increasing belowground C storage, in addition to mitigating atmospheric CO_2 increases, is important for maintaining nutrient cycling processes and soil biological activity (Raich and Schlesinger 1992).

R_S from forest ecosystems is closely linked to the type of organic substrate inputs in terms of quantity and chemistry (Samuelson et al. 2009). Thus any shift in forest management activity that arises from changes in species composition can affect decomposability from a heterotrophic perspective impacting C balance of the system. Understanding various biotic and abiotic controls such as species composition, roots, microbial biomass, soil type, microclimate and site preparation affecting soil respiration (Raich and Tufekcioglu 2000) are becoming increasingly important as changes in R_S responses to these variables can influence NEP as well as the rate of cycling of soil C to the atmosphere (Jenkinson et al. 1991, Schimel et al. 1994).

Biotic and abiotic controls on soil respiration

Vegetation Control

Vegetation is one of the most important drivers of R_S rates through root turnover inputs and root exudates that fuel soil microbial respiration and activity. Vegetation is a function of climatic factors such as solar radiation, temperature and moisture availability. The interplay between these factors is apparent as plant growth increases in the spring and summer as day length increases and temperatures rise and decreases in winter. R_S shows a strong correlation with temperature and NPP globally.

Annual R_S rates have been shown to be strongly and positively correlated with aboveground net primary production (NPP) with $r^2 = 0.87$ (Schlesinger and Andrews 2000) and with increasing rates of detritus production (Raich and Tufekcioglu 2000). A review paper published by Raich and Schlesinger (1992) examined published annual CO_2 flux data measured in different vegetation types. R_S rates are lowest in the tundra and northern bogs ($60 \pm 6 \text{ g C m}^{-2} \text{ yr}^{-1}$, $94 \pm 16 \text{ g C m}^{-2} \text{ yr}^{-1}$) which have the coldest climate and in the driest deserts ($224 \pm 38 \text{ g C}$

$\text{m}^{-2} \text{yr}^{-1}$), while the highest rates occurred in tropical moist forests ($1260 \pm 57 \text{ g C m}^{-2} \text{yr}^{-1}$) where both temperatures and water availability are high year-round. Temperate grasslands, temperate coniferous forests and temperate deciduous forests fell in between this range in terms of R_S rates, $442 \pm 78 \text{ g C m}^{-2} \text{yr}^{-1}$, $681 \pm 95 \text{ g C m}^{-2} \text{yr}^{-1}$ and $647 \pm 51 \text{ g C m}^{-2} \text{yr}^{-1}$ respectively. Primary productivity of forests is also greatest in tropics and declines with increasing latitude to low values in boreal forests and shrub tundra. In another study by Moore (1986), R_S rates were also shown to increase with increasing aboveground NPP within the northern peatland ecosystem, while another study has shown positive response of R_S rates in forest ecosystems to aboveground litter production (Raich and Nadelhoffer 1989). Such studies therefore also indicate a tight coupling between R_S and plant productivity, the latter of which correlate with temperature. Along a gradient of decreasing precipitation, NPP declines from forests to grasslands and is very low in most deserts. Although temperature is considered one of the principal driving factors of R_S , study done by Lloyd and Taylor (1994) measuring R_S rates in different locations under same temperature range showed large differences in rates attributing to variation in soil C composition. Soil C composition was largely considered to be influenced by longstanding vegetation.

Soil Temperature and Soil Moisture

On a global scale, R_S rates are shown to positively correlate with mean annual air temperatures and mean annual precipitation. Warmer temperatures and high precipitation together extend the growing season for plant growth which sustains the activity of soil microorganisms. Studies have shown temperature to be the principal factor driving R_S (Singh and Gupta 1977, Raich and Schlesinger 1992, Davidson et al. 1998) explaining anywhere up to 50% of the interannual variation. Moisture, though secondary to temperature, helps to increase

the predictive power of a model by interacting with temperature (Davidson et al. 1998). Moisture becomes increasingly important below or above a certain threshold in driving down R_S rates such as in very dry environments (deserts) where moisture is limiting and very wet or saturated environments (bogs) by inhibiting microbial activity.

(i) *Temperature*

Studies have documented strong relationships between R_S and soil temperature for loblolly pines as well as for other tree species (Maier and Kress 2000, Pangle and Seiler 2002, Samuelson et al. 2004, Pang et al. 2013). In a study by Wiseman and Seiler (2004) in loblolly pine plantation on the Virginia piedmont, CO_2 efflux rates closely paralleled soil temperatures, being the highest during the growing season (June to September) and lowest during the winter months. The sensitivity of R_S to temperature is also documented by the distinct diurnal pattern of R_S rates where the rates increase in the afternoon and decrease in the morning and night (Qui et al. 2005).

Rising temperatures accelerate the rate of microbial decomposition of soil organic matter (SOM) (Edwards 1975, Fenn et al. 2010), which is a source of microbial metabolism, thereby increasing R_S rates and resulting in faster turnover rate of C from soil to atmosphere. Fenn et al. (2010) studied the effects of temperature on total soil CO_2 efflux and on the three partition coefficients making up the total R_S : soil organic matter (R_{SOM}), root rhizosphere (R_{RHIZ}) and mycorrhizal (R_{MYC}) components in a temperate deciduous forest. Experimental manipulations using mesh bags of different sizes were carried out to (i) exclude roots but allow the growth of mycorrhizal hyphae, (ii) and exclude both roots and hyphae. Mesh bags were placed *in situ* in the ground and open top collars were installed in the center of each bag to allow entry of litterfall as

on unpartitioned soil. R_S was measured from April to November using an infrared gas analyzer on the mesh collars and over regular collars without any manipulation, and relative contributions of R_{SOM} , R_{RHIZ} and R_{MYC} of total R_S was determined. Total R_S followed temperature significantly ($P < 0.0001$), increasing in spring to summer and declining in winter. Soil water content had a less clear pattern with R_S than temperature. All three components showed positive relationship with temperature and solar radiation, but only R_{RHIZ} was significant with temperature, while R_{RHIZ} and R_{SOM} were significant with insolation. R_{RHIZ} and R_{SOM} also decreased with increasing soil water content, while R_{MYC} showed a positive relationship. R_{SOM} was significantly higher than R_{MYC} throughout the 11 weeks, but significantly higher than R_{RHIZ} for half the period only. Even though R_{SOM} was significantly higher, it was still believed to be an underestimation due to absence of roots leading to lack of root exudates which soil microorganisms respire for their metabolism. Therefore, current concern with climate change on soil C dynamics is that increased temperatures could increase the R_{SOM} component driving soil respiration which could alter carbon sequestration dynamics.

In a field study conducted by Davidson et al. (1998) in a temperate mixed hardwood forest, R_S rates kept increasing over summer until the effects of natural summer drought caused soil moisture levels to fall below a level that was limiting to R_S . Mid-September rain again increased R_S rates by relieving water stress, but not to levels as high as predrought levels.

Laboratory studies have shown microbial biomass C to increase at higher temperatures. In a laboratory study conducted by Qui et al. (2005) where R_S was measured on soils incubated at 20°C and 31°C, both of which were treated with leachate and rainwater, R_S rates more than doubled from $175 \pm 28 \text{ mg m}^{-2} \text{ h}^{-1}$ and $365 \pm 63 \text{ mg m}^{-2} \text{ h}^{-1}$ at 20°C and 31°C respectively. This temperature sensitivity is expected to be higher in lower temperatures (such as the boreal

peatlands and tundra if they were to also experience any water table drop) than warmer temperatures (Lloyd and Taylor 1994).

(ii) *Moisture*

The influence of soil temperature on R_S is modified by soil moisture (Wiseman and Seiler 2004). Soil saturation can impede diffusion of oxygen, which slows down the activity of microbes involved in decomposition and CO_2 production. Laboratory experiments on the effects of varying levels of soil water content on R_S rates has shown R_S rates to decrease after a certain moisture threshold attributing to dormancy or death of microorganisms via desiccation, slowing enzyme reaction rates and limiting rate of solute diffusion to enzyme sites (Wildung et al. 1975, Wilson and Griffin 1975, Wilson and Harris 1968, Orchard and Cook 1983, Skopp et al. 1990) and by reducing contact between microorganisms and available substrates (Yu et al. 2011).

As opposed to temperature, R_S studies on temperate forests including loblolly pines have found weak correlations between R_S and soil moisture where soil moisture impacts R_S only at extremes (very dry or very wet) (Maier and Kress 2000, Pangle and Seiler 2002, Samuelson et al. 2009). Davidson et al. (1998) conducted a field study on the Prospect Hill tract of the Harvard Forest during a drought-struck year to particularly study the effects of low soil water content on R_S rates by taking advantage of the summer drought of June 1995. R_S declined most rapidly on well drained site followed by poorly drained and lastly by very poorly drained site (where most moisture was present). Mid-September rain again caused R_S to increase in all three sites, but it eventually declined as temperatures decreased. This shows that although temperature seems to be the primary driver of R_S , moisture becomes important at extremes.

Orchard and Cook (1983) examined the relationship between microbial activity and water potential in a silt loam soil where temperature was kept constant at all times. Microbial activity was inferred from the rate of CO₂ evolution or soil respiration. Soils were collected from a grazed pasture land, sieved to remove roots, air dried (evaporation) and rewetted to a range of water potentials, -0.005, -0.01, -0.05, -0.4, -1.0 and -1.5 MPa. They observed a log-linear relationship between respiration and water potential with microbial activity being higher in wetter samples (-0.005, -0.01, -0.05 MPa) than drier samples (-0.4, -1.0, -1.5 MPa). Their results well align with the results of Wilson and Griffin (1975) who studied microbial respiration from forest soils incubated in a sealed chamber at different water potentials. They also observed marked increases in microbial respiration with increasing (less negative) water potential. Respiration was studied by the amount of oxygen consumed from a sealed respiration chamber. Soil reaching near saturation having very little air-filled pore space and this has been shown to depress microbial activity by limiting the supply of O₂ (Rovira 1953).

Soil CO₂ efflux partitioning

Ecologists and biogeochemists have developed a conceptual framework for defining the various components contributing to C fluxes from an ecosystem (Schlesinger 1997). Gross primary production (GPP) is the amount of C assimilated by photosynthesis. Net primary production (NPP) is the net accumulation of C per unit of land.

Below are some of the key relationships in terrestrial ecosystems:

- (i) $NPP = GPP - R_P$ (where R_P is total plant respiration)
- (ii) $NEP = NPP - R_H$ (where R_H is heterotrophic respiration)
- (iii) $R_S = R_A + R_H$ (where R_A is autotrophic respiration)

Knowing the R_A and R_H component is necessary in finding the net ecosystem productivity (NEP). NEP, which is modeled by subtracting R_H from NPP is used to estimate C budgets at regional and continental scales. It includes all C exchanges that result in net C accumulation by an ecosystem (Randerson et al. 2002) and indicates whether an ecosystem functions as a net source or sink for atmospheric CO_2 (Maier and Kress 2000). The above NEP equation however is ignoring other losses occurring from respiration of herbivores (which is negligible), losses from fire, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and volatile organic compounds (Schlesinger 1997).

The partitioning of R_S helps to improve our understanding of the different environmental controls that drive the two components (Bond-Lamberty et al. 2004) and to accurately estimate C budgets. While plants store significant amounts of atmospheric C belowground in the roots (Schlesinger 1984) which enters the soil, plants are important autotrophic component of R_S releasing CO_2 via root respiration. R_A contribution increases in summer as photosynthate production and transport to roots increase root production (Gaumont-Guay et al. 2007).

Estimating relative components of R_A and R_H accurately has been one of the most difficult challenges in the efforts to fully quantify the C cycle (Kuzyakov 2006). But accurate estimates of the two R_S components are required as they are functionally different processes and show variation spatially and temporally with species composition, temperature, moisture, NPP, soil type and land management activities (Boone et al. 1998, Rustad et al. 2000, Gaumont-Guay et al. 2008, Lamberty et al. 2011, Guay et al. 2007). Raich and Tufekcioglu (2000) reported that root contribution to R_S was 33-89% in forests, 17-40% in grasslands, 12-38% in croplands, and 50-93% in arctic tundra. R_A alone is estimated to contribute 30 to 60% of the total R_S from broad-leaf and pine forests in temperate zones (Raich and Tufekcioglu 2000). Hanson et al.

(2000) reported a range of 10-90%, wider yet, for the annual contribution from R_A in forest stands.

Hogberg et al. (2001) used girdling of trees to study partition of R_S in a boreal Scots pine (*Pinus sylvestris*) forest in Sweden. Tree girdling involves girdling of phloem which interrupts the flow of photosynthates from leaves to roots leading to suppression of root and rhizomicrobial respiration. They reported a decrease of 27% in R_S in plots that were girdled in June within 5 days relative to control R_S and further 52% decrease in those same girdled plots by the end of 9 weeks. Late girdling plots (plots girdled in August) showed an even faster decline relative to control plots, 37% within five days of girdling and 56% by the end of two weeks. The late girdled plots took less time to reach the similar equilibrium as plots girdled in June suggesting that, under the same experimental conditions, R_A contribution to R_S varies with the time of the year within a site.

Another study done on *Picea mariana* in Canada showed tree root respiration which was calculated based on plant belowground C allocation and estimates of root growth to comprise only 24% of total soil respiration (Malhi et al. 1999). Wiant (1967) summarized evidence suggesting that root respiration comprises at least one-third of total soil CO_2 efflux in forests annually. Furthermore, Bowden et al. (1993) has identified studies which have reported relative contribution from live roots to be 35% from a 50-year-old tulip-tree (*Liriodendron tulipifera* L.) forest in Tennessee, while also making note of other studies which reported root contribution of 62% from a 29-year-old slash pine (*Pinus elliottii* Engelm) plantation in Florida and 47-51% from an 80-year old pine (*Pinus densiflora*) forest in Japan. Bond-Lamberty et al. (2011) modeled R_A from R_S and reported an annual root contribution of 33% from 15-year old boreal forest of black spruce stand in Canada. These studies show that live root respiration can be a

major contributor to total R_S accounting anywhere from one- to two-thirds of annual C release from forest soils. In temperate woodlands, both root respiration and heterotrophic respiration show seasonal variation, but both showed the greatest fluxes in the growing season (Savage and Davidon 2001).

Estimating the contribution of autotrophs and heterotrophs will allow us to get a good handle on how the individual components respond differently to various environmental and management influences and how they may influence atmospheric CO_2 concentrations relative to one another. A study by Scott-Denton et al. (2006) examining the interplay of climate and substrate factors on the components of soil respiration found that while rhizospheric respiration (composed of roots, mycorrhizal fungi and microorganisms that subsist on root exudations) was largely influenced by available C from the shoots and was hence associated with productivity, heterotrophic respiration (free-living decomposers of soil organic carbon) seemed to be more strongly influenced by soil moisture conditions.

Trace gas fluxes: CH_4 and N_2O

Soils store the largest amount of C and N found in terrestrial ecosystems. In addition to CO_2 , soils are important sources of CH_4 and N_2O gases which are produced as a result of microbially mediated biochemical reactions. Roughly a third of CH_4 emissions and two-thirds of N_2O emissions to the atmosphere come from soils (Smith et al. 2003).

Methane

The global mean atmospheric CH_4 concentration in 2005 was 1.732 ppm (relative to 379 ppm for CO_2) which far exceeds the pre-industrial values determined by ice core data of 0.3 to

0.7 ppm (Denman et al. 2007). Although, atmospheric CH₄ concentrations have more than doubled over the last century, measurements made in the early 2000s by Dlugokencky et al. (2003) and Simpson et al. (2006) showed slowdown in the rate of CH₄ increase to near-zero.

The global pool of atmospheric CH₄, which is the second most important anthropogenic greenhouse gas behind CO₂, is in the order of 500-600 Tg CH₄ yr⁻¹ (Wang et al. 2004). Wetlands are a significant natural source of atmospheric CH₄ emission with most studies focusing on the northern boreal wetlands in this context (Grand and Gaidos 2009). CH₄ is formed in soils by the microbial breakdown of organic compounds in strictly anaerobic conditions at a very low redox potential (Smith et al. 2003). CH₄ is produced by methanogenic archaea either by fermenting acetate (CH₃COOH) into CH₄ and CO₂ (CH₃COOH → CO₂ + CH₄) or by CO₂ (present in the form of HCO₃⁻) reduction in presence of hydrogen to yield CH₄ and water (CO₂ + 4H₂ → CH₄ + 2H₂O (Schlesinger 1997). Production of CH₄ does not begin until reduction of molecular oxygen, nitrate, iron (III), manganese (IV) and sulfate is complete (Smith et al. 2003). Loss of CH₄ from soils is the difference between production by methanogenic archaea in anoxic zones at depth, and aerobic methane oxidation by methanotrophic bacteria as it diffuses up through zones of higher redox potential (Grand and Gaidos 2010).

The current major sink for atmospheric CH₄ is reaction with hydroxyl radical in the stratosphere (approx. 450 Tg yr⁻¹) (Wang et al. 2004) where OH radicals oxidize CH₄ to form CO₂ and water vapor. The only known biological atmospheric CH₄ sinks are microorganisms in terrestrial environments (Stuedler et al. 1989). The shift from flooded wetland soils to well drained upland soils has been shown to result in a shift from net emission of CH₄ to net uptake from the atmosphere (Bartlett and Harris 1993).

Aerobic soils in temperate forests have been found to be the largest biological sink for atmospheric CH₄ (10 Tg yr⁻¹) through microbial oxidation (Adamsen and King 1993, Castro et al. 1995, Bartlett and Harris 1993). CH₄ diffuses from the atmosphere into upland soils in small amounts where it is oxidized by methanotrophs and nitrifying bacteria with methanotrophs having a greater ability to oxidize CH₄ relative to nitrifiers (Castro et al. 1994). CH₄ is utilized by methanotrophs as a source of reduced C for energy (Zehnder and Brock 1979) by means of an oxygenase, an enzyme which requires oxygen to carry out this process (Higgins and Quayle 1970). About 30 Tg of atmospheric CH₄ entering the soil is oxidized to CO₂ by aerobic soil bacteria which are adapted to live on small concentrations of this substrate (Smith et al. 2003). Higgins and Quayle (1970) have conducted laboratory experiments by growing *methanooxidans* in presence of labeled oxygen (¹⁸O₂) or labeled water (H₂¹⁸O). Results indicated negligible incorporation of water derived oxygen into methanol, which is the first molecular compound that is formed during oxidation of CH₄. The oxygen atom in methanol formed by oxidation of CH₄ derived from molecular oxygen and not from water.

CH₄ consumption in temperate and tropical forest soils has typically known to range from 1.0 to 5.0 mg CH₄ m⁻² day⁻¹, with lower values after rainstorms which reduce diffusion rates of O₂ and CH₄ in soil matrix (Adamsen and King 1993, Bartlett and Harris 1993). Schmer et al. 2012 have reported negative CH₄ fluxes down to -384 μg CH₄ m⁻² d⁻¹ and positive fluxes up to 240 μg CH₄ m⁻² d⁻¹ for fertilized switchgrass in North Dakota. In desert soils where the supply of labile organic matter is limited, soil bacteria consume an average of 0.66 mg CH₄ m⁻² d⁻¹, with the greatest rates observed after rainstorms opposite to the response of temperate forest soils (Striegl et al. 1992). While temperature is the important determinant of CO₂ production,

important controls on methane are soil water content and diffusive gas transport within the soil matrix (Adamsen and King 1993, Smith et al. 2003).

Some soil nitrifying bacteria have been found to use CH₄ as an alternative substrate to ammonium (NH₄); however, their consumption toward CH₄ is observed to be lower in forests receiving large amounts of NH₄ either from atmospheric deposition or N fertilization (Adamsen and King 1993, Castro et al. 1994). Adamsen and King (1993) studied the effects of various inorganic N sources on CH₄ consumption via laboratory soil incubation and found that all NH₄ treatments significantly decreased CH₄ uptake rates by 61 to 95% than a comparable concentration of sulfate salt. In another study by Castro et al. (1994) where CH₄ fluxes were measured from control and 4 years of urea-fertilized soils in a mature slash pine plantation, they observed 5–20 times lower daily mean CH₄ consumption (0.001-0.007 mg CH₄-C m⁻² h⁻¹) in fertilized soils relative to control soils (0.015-0.035 mg CH₄-C m⁻² h⁻¹) after which they concluded that additions of ammonium can inhibit CH₄ consumptions in mineral soils. Previous studies conducted by Mosier et al. (1991) and Steudler et al. (1989) have also reported short-term and long-term significant decreases in atmospheric CH₄ consumption due to N fertilization, while increasing N₂O production from the system.

Nitrous oxide

Nitrous oxide (N₂O), which is the third most important contributor to current radiative forcing, has increased by about 16% from its pre-industrial level of 270 ppb to 319 ppb in 2005 (Denman et al. 2007). Global N₂O emission is estimated at 17.1Tg N yr⁻¹, with soil emissions contributing to 36% (Schlesinger 2013). There is growing concern about the flux of N₂O as its concentrations in the atmosphere are increasing almost linearly at an annual rate of 0.26% for the

last several decades (Denman et al. 2007). Such small atmospheric increases can have long lasting effects as N₂O has an atmospheric residence time of 100-175 years. N₂O consumed in photochemical reactions in the stratosphere is the only significant sink of this gas, but which results in ozone depletion (Knowles 1982), while few soils also appear to consume N₂O (Donoso et al. 1993).

Soils are an important source of N₂O which is formed as a result of two microbial processes: nitrification, the aerobic conversion of NH₄ to NO₂ and then to NO₃, and denitrification, which involves reduction of NO₃, to atmospheric N₂ releasing N₂O as an intermediate product (Basiliko et al. 2009, Bollman and Conrad 1998). Nitrification is an aerobic process, but when supply of O₂ is limited the nitrifying bacteria can use NO₃ or NO₂ as an electron acceptor and reduce it to NO and N₂O (Bollman and Conrad 1998).

Heterotrophic activity is still present in anoxic conditions, when soils or microsites within the soil profile may be waterlogged, with NO₃ or NO₂ serving as the terminal electron acceptor in metabolism. NO₃ readily acts as an electron acceptor for microbial respiration once O₂ is exhausted so denitrification, unlike CH₄ production, can occur rapidly (Smith et al. 2003). The net flux of soil N₂O to the atmosphere results from the activity of nitrifying and/or denitrifying bacteria producing N₂O, and its consumption due to reduction of N₂O to N₂ gas.

Aerobic soils in the temperate zones are known to be both potential sources and sinks for N₂O. Forest soils, unlike agricultural soils, have been reported to act as sink by several studies, with N₂O uptake rates ranging from 0.83 to 6.7 µg N₂O-N m⁻² h⁻¹ (Castro et al. 1993, Goosens et al. 2001). Although only a small fraction of N is lost as N₂O during nitrification and denitrification, the rise in N₂O concentrations in the atmosphere has important implications for potential greenhouse warming and ozone destruction in the stratosphere (Schlesinger 1997).

The addition of NO_3 slows N_2O uptake in soils (Blackmer and Bremner 1976, Bremner 1997). N enrichments in terrestrial ecosystems through increasing rates of fertilizer application has shown to stimulate nitrification and denitrification rates taking place in soil which are linked to rapid rise in atmospheric N_2O concentrations (Vitousek 1994). Matson et al (1987) found a direct relation of N_2O production and N mineralization in various tropical forests, linking N_2O production to nitrification process, but in the wet soils of Amazon rainforests, N_2O appeared to be the mainly from denitrification (Keller et al. 1988). N_2O flux from soil to atmosphere is increased due to higher rates of nitrification which can be attributed to fertilization of soils (Halverson and Grosso 2011).

METHODS

Study Site

This study site was located near Dover on the Lower Coastal Plain of North Carolina (35°15'N, 77°28'W) with a mean annual temperature of 17.3°C, and mean annual precipitation of 1259 mm (US Climate Data). The research site was a 28 ha field experiment established in 2009 and maintained by Catchlight Energy LLC, a joint venture between Chevron Corporation and Weyerhaeuser Company. This large, interdisciplinary experiment is examining a loblolly pine (*Pinus taeda* L.) dominated plantation intercropped separately with warm season perennial switchgrass (*Panicum virgatum*). The soils are mapped as the Pantego (fine-loamy, siliceous, semiactive, thermic Umbric Paleaquults) and/or Rains series (fine-loamy, siliceous, semiactive, thermic Typic Paleaquults) which are very deep, poorly drained, moderately permeable soils (Albaugh et al. 2012). The soil is medium- to coarse-textured (sandy loam to sandy clay loam) and consists of approximately a 0-18 cm deep organic layer. The soil information is based on pre-ditching conditions of the site. The site was partially drained in the 1970s through ditching to lower the water table and subsequently improve hydrologic conditions for pine establishment and growth (Albaugh et al. 2012).

The study design is a completely randomized block with seven treatments replicated across four blocks (Fig. 1). Our specific research only examines three treatments within this larger study design: (1) traditional bedded pine (pine with no switchgrass), (2) flat planted switchgrass (SG), and (3) bedded pine intercropped with switchgrass in the interbed space (PSG) (Fig. 2). Three different locations were measured within PSG treatment: (i) on the bedded pine row (PSG-B), (ii) in the middle of the switchgrass planted in the interbed (PSG-I), and (iii) on the edge, or transitional boundary between pine and switchgrass (PSG-E); and two in the

traditional bedded pine treatment: on the bedded pine row in P (P-B), and in the interbed of the bedded pine rows (P-I). Thus, there were six separate microsites (Fig. 2) utilized in this study (i.e., P-B, P-I, PSG-B, PSG-E, PSG-I, and SG) to provide different contrasts based on our stated objectives. Each treatment plot is 0.8 ha in size with a 0.4 ha measurement plots, surrounded by a 15 m buffer. Stand age is uniform across treatments and blocks.

Loblolly pine trees were planted in the winter of 2008 and switchgrass (cultivar Alamo) planted in summer 2009. Prior to planting, different site preparation methods were undertaken depending on specific treatments. These included V-shearing and bedding in the P-B and PSG plots and V-shearing and root raking in SG plots. Non-merchantable biomass, specifically any coarse woody debris (CWD) greater than 5 cm was removed by grapple-claw excavator and piled along the edges of the intercropped plots, while V-shearing and root raking removed most harvesting residuals from switchgrass plots (Albaugh et al. 2012). All pines trees were hand planted in straight bedded rows at 1100 trees ha⁻¹ (435 trees ac⁻¹) and a spacing distance of 6 m was maintained between rows. Switchgrass was machine planted at 9 kg pure live seed ha⁻¹ in rows spaced 40 cm apart to a depth of 0.6 cm and covered with soil (Albaugh et al. 2012). Weyerhaeuser's proprietary liquid suspension-based fertilizer comprising of 3% N, 6.2% phosphorus (P), 2.5% potassium (K), 4.5% magnesium (Mg) and 2% calcium (Ca) was applied into beds and between each row of switchgrass during planting to promote seedling growth and establishment (Albaugh et al. 2012).

Switchgrass was fertilized in the second growing season (June 2010) using Weyerhaeuser's coated Arborite fertilizer, supplying 65.6 kg N ha⁻¹, 6.6 kg P ha⁻¹ and 0.2 kg B ha⁻¹ (Albaugh et al. 2012). During this period, switchgrass plots were sprayed with 2, 4-D (4.68 l ha⁻¹) and Basagran (0.88 l ha⁻¹), post-emergent herbicides (Albaugh et al. 2012) to control

competing vegetation. Imazapyr (pre-emergent herbicide) was used to control competition before planting pine seedlings. Switchgrass was fertilized again in April 2012 with the aforementioned liquid suspension fertilizer. Switchgrass was harvested in December 2011 during the course of our sampling period and in late March in 2013 after sampling ended.

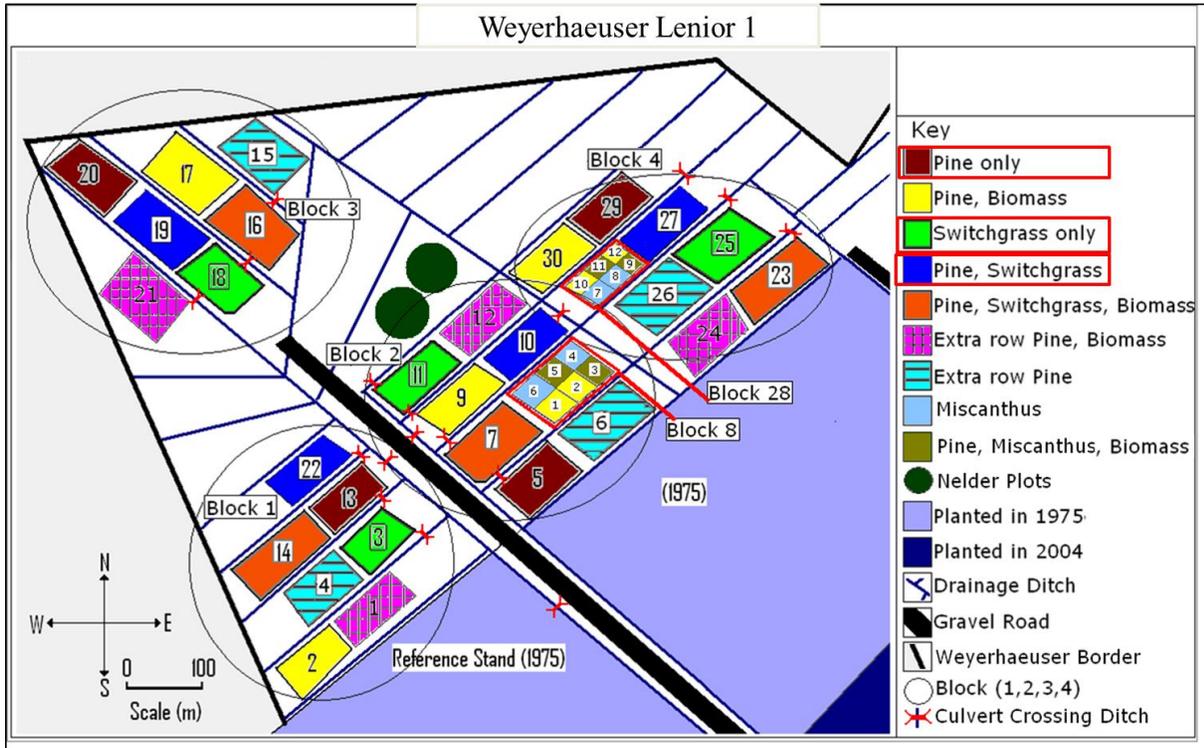


Figure 1. Design and plot layout for the Catchlight Energy LLC (Chevron Corporation and Weyerhaeuser Company joint venture) switchgrass loblolly pine intercropping study located near Dover, NC. Treatments within the red boxes are treatments under consideration.

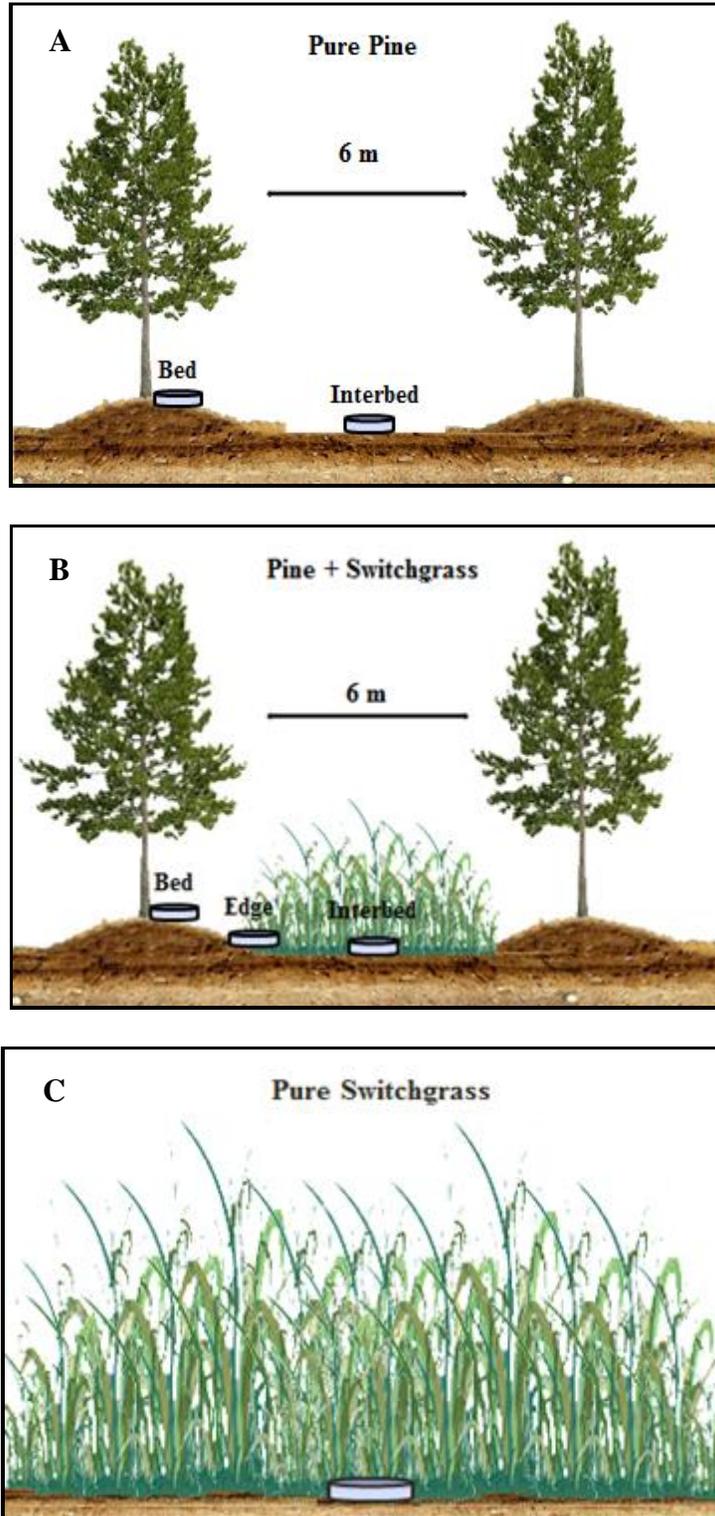


Figure 2. Main treatments measured and different microsite sample locations where measurements were made: (A) pine: bed (P-B), interbed (P-I); (B) pine + switchgrass: bed (PSG-B), edge (PSG-E), interbed (PSG-I); (C) switchgrass (SG).

In situ soil CO₂ efflux

R_S was measured at roughly 6-week intervals from January 2012 to March 2013 using a portable LI-6200 infrared gas analyzer (Li-Cor, LI-6200, Lincoln, NE, USA) equipped with a Li-Cor 6000-09S chamber with a 926 cm³ volume covering 72 cm² of soil surface. The gas analyzer was zeroed and spanned with 359 ppm CO₂ reference prior going to the field. Three subsample locations were measured from each block in five of the microsite locations. All measurements were conducted in daylight between 9 am and 4 pm, one block at a time. The CO₂ concentration in the cuvette chamber was allowed to come to equilibrium with the ambient CO₂ concentration near the soil surface which generally ranged between 370 and 430 ppm (Tyree et al. 2006). A vegetation free spot was chosen to place the chamber, with the exception of mosses which sometimes covered the forest floor beneath the litter layer, and care was taken to ensure a good seal between the chamber and soil surface. Sampling began when CO₂ concentrations steadily rose for at least a 30 second period. Soil temperature at a depth of 7.5 cm using a digital thermometer and percent volumetric soil water content across 12.0 cm depth using a Hydrosense meter (Campbell Scientific, Logan, Utah, USA) was taken concurrently in each of the sample locations.

Measurements were conducted from each plot of the three treatments:

1. Pine bed (P-B)
2. Switchgrass (SG)
3. Three different microsites in the pine + switchgrass intercrop (Fig. 2):
 - a. Bedded pine (PSG-B)
 - b. Pine + switchgrass interface (PSG-E)
 - c. In the middle of switchgrass (PSG-I)

Partitioning of autotrophic and heterotrophic respiration

A technique based on root carbohydrate depletion (Bond-Lamberty et al. 2011) was used to partition autotrophic and heterotrophic respiration by installing 35cm long, 10 cm inside diameter steel cores into the ground. The deep coring severs the roots from their carbohydrate source, ideally eliminating root respiration over time inside the cores. The first set of deep cores was installed in March at the three microsites in the PSG treatment: PSG-B, PSG-E, and PSG-I. Three subsamples were randomly located at each of the microsite types. R_S was measured on the exact spot prior to core installation and four times (roughly every 6 weeks) over a period of 100 days after installation directly over the exclusion cores, and immediately adjacent to the core. Aboveground vegetation that had grown inside the cores was clipped before taking a respiration measurement. A total of 36 cores were installed (3 treatments \times 3 subsamples \times 4 blocks). As described above, soil temperature at a depth of 7.5 cm using a digital thermometer and percent volumetric soil water content over 12 cm were also measured. Soil moisture and temperature was not measured inside the deep cores to avoid disturbing the system. However at the final sample date, prior to destructive sample harvest, soil moisture and soil temperature were taken directly inside the cores.

After a period of root carbohydrate depletion, the soil CO_2 efflux measured inside the cores was used as a proxy for R_H . The proportion of R_S attributed to R_H was then calculated by dividing the flux rate from inside the cores by the flux rate from outside the cores (R_H/R_S).

The cores were extracted in July after 100 days and returned to the lab for later analysis and root sampling. A new set of cores were installed in August and harvested in November after 98 days, for two separate estimates of R_H . However because of low soil temperatures, R_S rates were very low by the time cores were scheduled to be removed in November 18, 2012. This made

partitioning R_S difficult due to the low efflux rates in the context of our analytical precision. Thus, for this period of our R_S partitioning, we chose to use the previous measurement date [October 26, 2012 (day 73)] for our R_H calculation.

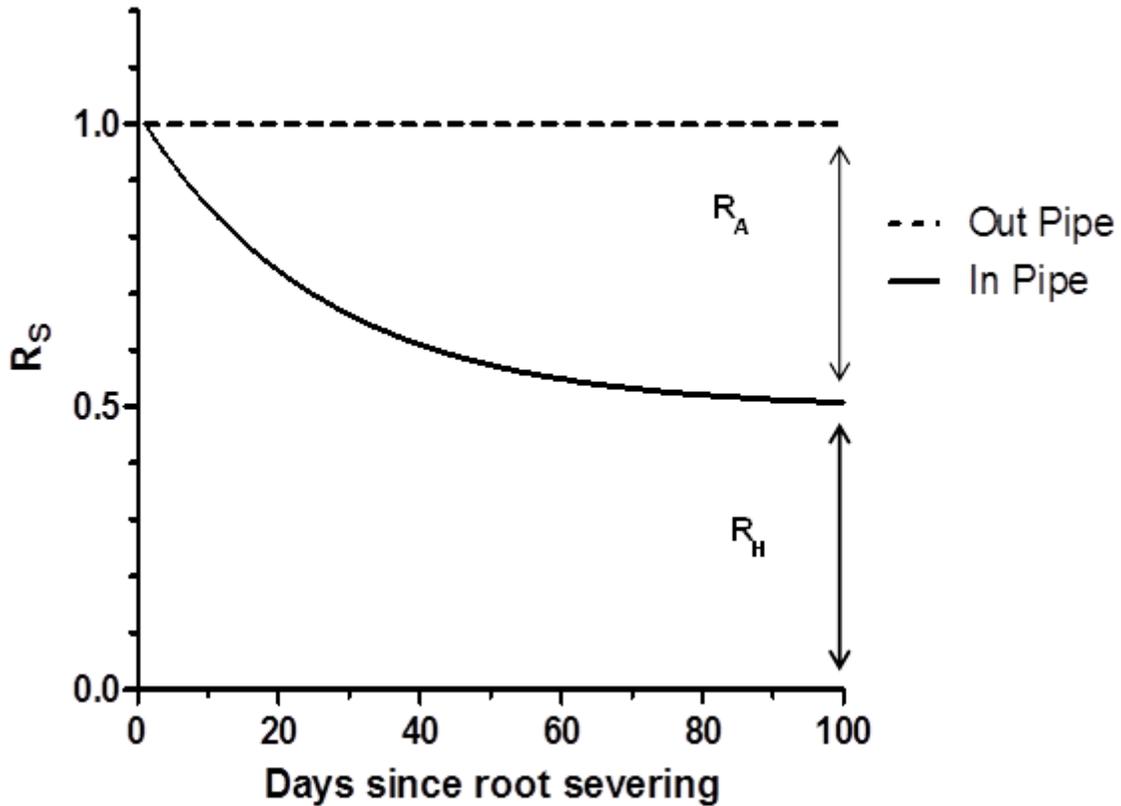


Figure 3. Hypothetical response in soil respiration (R_S) over time to root severing where the autotrophic component of soil respiration (R_A) declines as root carbohydrate supply is diminished resulting in heterotrophic respiration (R_H) as the sole source of R_S within the root exclusion cores (pipes).

Figure 3 is a hypothetical response demonstrating the partitioning of R_S into R_H and R_A . The deep core isolates a volume of soil severing the roots from their carbohydrate supply. As carbohydrates are depleted in the isolated roots, root respiration (R_A) gradually decreases. Total R_S over the deep core is expected to decrease over time, eventually reaching an asymptote relative to R_S (measured immediately outside and adjacent to the collar) at which point the assumption is that the CO_2 efflux is due to R_H alone.

Root Analysis from exclusion cores and fresh cores

Following the last CO_2 efflux measurement the deep soil cores were removed and soil was collected for subsequent root analysis. Fresh soil cores were also taken immediately adjacent to the root exclusion cores at the exact location where soil CO_2 efflux measurements outside the pipe had been recorded. In addition, fresh soil cores were extracted from P-B and SG treatments (3 subsamples per treatment plot). All cores were separated into 0-15 cm and 15-35 cm depth increments. Similarly, roots from the second set of root exclusion cores were collected; however, these cores were not separated into separate depth increments and additional cores (e.g., fresh soil, other micro-topographical positions) were not taken.

Soil from extracted cores was washed through a 1 mm mesh screen. The mixture of roots, coarse woody debris and any solid particulates left behind on the sieve were collected. Roots were sorted into loblolly pine roots and switchgrass roots. Other “grass-like” roots obtained from P-B, where no switchgrass was known to be planted or observed aboveground, were categorized as “other” herbaceous grass roots. Coarse woody debris was collected, oven dried at $60^\circ C$ for at least 72 hours and weighed. WinRhizo (Regent Instruments, Inc.), image analysis software was used to scan roots for projected root length (cm) and root surface area (cm^2) for different root

diameter size classes (<1mm, 1-2 mm, 2-4 mm, >4mm). After scanning, the roots were oven dried at 60°C for 48 hours and weighed for determination of root biomass. Final root parameters are expressed per volume basis (e.g., root biomass in g m^{-3} , root length in cm dm^{-3} , and root surface area in $\text{cm}^2 \text{dm}^{-3}$).

Measurement of trace gas exchange of CH₄ and N₂O

A vented static chamber method was used for measurement of trace gas exchange between the soil surface and atmosphere (Hutchinson and Livingston 2001). The chambers were constructed of polyvinyl chloride (PVC) with dimensions 18 cm height and 25.4 cm inner diameter. One end of the chamber was beveled to facilitate insertion into the soil. The chambers were installed to a depth of 5-10 cm (Hutchinson and Livingston 2001) at least one month prior to taking the first flux measurement in order to minimize the effects of installation disturbance on flux rates. Subsequent samples were taken in August, November and February 2013 to capture seasonal variability. Once installed, the chambers were left in the field for the whole experimental period with the exception of the PSG-I treatment where chambers were temporarily removed in December 2012 to accommodate machinery associated with the switchgrass harvest. These chambers were reinstalled to the exact same location in January. Two microsite locations were sampled from each of the treatments: the bed and interbed of pine (P-B and P-I respectively) and pine switchgrass intercrop (PSG-B and PSG-I).

At the time of sampling, a PVC lid containing a gas sampling port equipped with a butyl rubber septum and vent tube (22 cm length, 1.1 cm inner diameter) which allows equilibration of internal and external atmospheric pressures was used to enclose the chamber. An instantaneous measurement was taken immediately upon sealing of the chamber head (initial or time-zero concentration) with a 20 ml polypropylene syringe fitted with one-way stop-cock valve.

Headspace gas samples were withdrawn from the chamber at regular intervals over a period of 60 minutes (e.g., 0, 20, 40, 60 min). Air samples were immediately transferred to a pre-evacuated glass vial sealed with butyl rubber septum that had previously been purged using N₂ gas and evacuated in the laboratory a minimum of three times after sealing, in order to minimize cross contamination. Soil temperature at 7.5 cm and volumetric soil moisture across 12 cm was also recorded. Glass vials were then transported to the laboratory and analyzed for CO₂, CH₄ and N₂O concentrations on a customized Shimadzu GC-2010 gas chromatograph (GC) equipped with an electron capture detector (ECD), flame ionization detector (FID), and methanizer. Soil surface trace gas fluxes were determined by calculating the rate of change of trace gas concentration over time. All measured concentrations, originally in ppm, were converted to mass units and corrected to field conditions (i.e., barometric pressure at 1 atm, air temperature during time of measurement) based on which fluxes were calculated (Holland et al. 1999).

Measurements were conducted in the bed and interbed microsites of:

1. Pine
2. Pine + switchgrass intercrop

Statistical Analysis

The average of subsamples in each plot was used as the experimental unit. The effects of treatments on soil CO₂ efflux, soil temperature and soil moisture were analyzed using Repeated Measures (Proc mixed model) analysis in SAS 9.2 software (SAS Institute, Cary, NC). Differences in treatments means were tested using Tukey-Kramer Honestly Significant Difference (HSD). The response of soil CO₂ efflux to environmental parameters was tested using multiple regression analysis. Further, independent models were created for each treatment to test whether the sensitivity of R_S to environmental variables like soil temperature and soil moisture differed among the five treatments. First, a stepwise regression method was used in JMP Pro 10 to select only those environmental variables that would best describe the R_S model across the entire data set. Variables that were included in the regression model at the starting point were R_S, soil moisture and soil temperature, various transformations of each variable (e.g., natural log, square root and inverse) and soil moisture by temperature interactions. Soil moisture and the interaction factor between soil moisture and temperature did not have significant effects on R_S and were removed from the model. The model for the relationship between log transformed R_S and soil temperature provided the best fit. Once this model was selected, slopes and intercepts of the models were tested for treatment differences using analysis of covariance.

Treatment effects on N₂O and CH₄ fluxes, and various root parameters in the three different depth categories (0-15 cm, 15-35 cm and 0-35 cm soil depth) were studied using one way ANOVA and statistical analyses were carried out on JMP Pro 10. When necessary, data transformations were carried out in the form of log and square root transformation to fulfill the assumption of normality and equal variance required by ANOVA. R_H was calculated for each subsample from the efflux values at day 100 and 73 in summer and fall respectively and then

averaged per plot. Treatment differences in log transformed R_H were analyzed by one-way ANOVA. Spearman's correlation was carried out between root parameters and associated R_A and R_S . Spearman's correlation was run for each of the different depth category from which roots were collected (0-15cm, 15-35 cm and total depth). Only the depth(s) showing significant correlations were included.

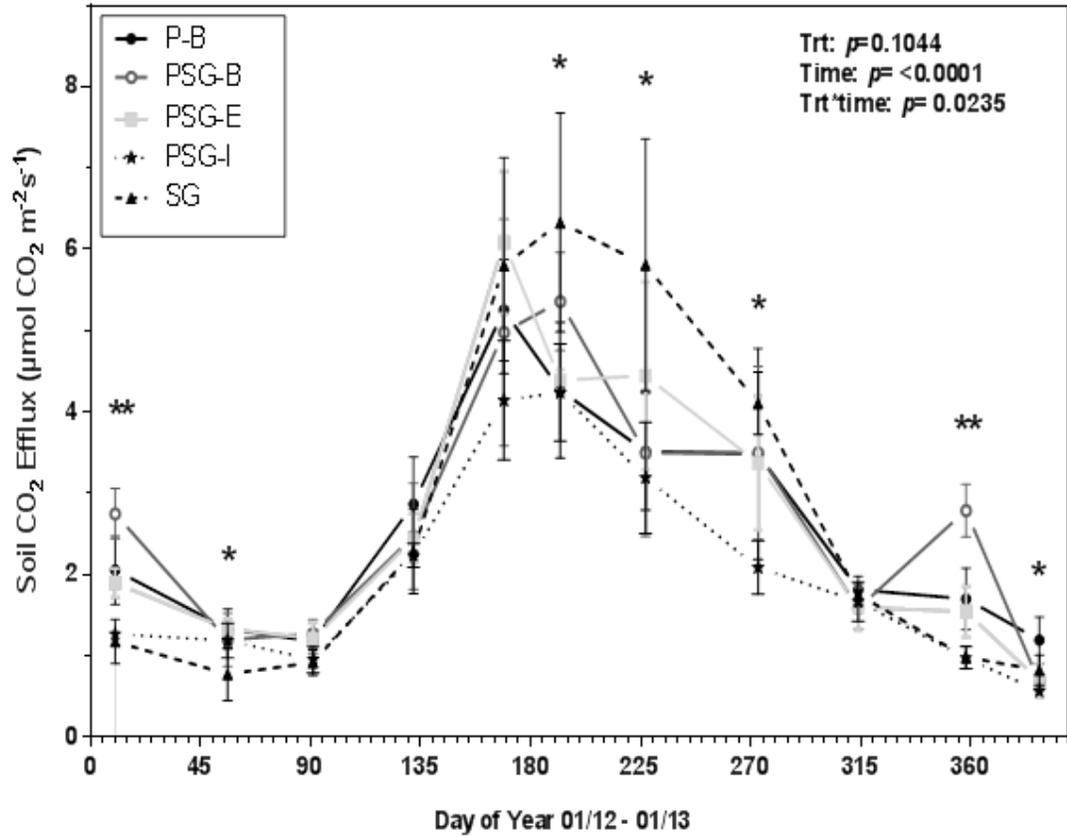
RESULTS

Soil CO₂ efflux

Treatment effects on soil CO₂ efflux, soil temperature and soil moisture

R_S showed a strong seasonal pattern (Fig. 4) that closely followed patterns in soil temperature (Fig. 5). The highest R_S rates occurred during the summer months in SG (Fig. 4) where significant differences between SG and PSG-I were observed during July ($p = 0.0793$), August ($p = 0.0859$) and September ($p = 0.0934$) (Fig. 4). R_S did not significantly differ consistently between other treatments at other times of the year, although notably R_S was lower in the presence of switchgrass (SG and PSG-I) in some winter months (January and December 2012) (Fig. 4).

Soil temperature and moisture showed dramatically different patterns. Soil temperature exhibited strong seasonal patterns, but few treatment differences (Fig. 5). Conversely, soil moisture did not show seasonal trends, but was lower on every sampling date on the elevated beds (P-B and PSG-B) relative to the PSG-E, PSG-I or SG treatments (Fig. 6).



	Jan10	Feb25	Mar30	May10	Jun16	Jul10	Aug14	Sep15	Oct26	Dec09	Jan19
P-B	b	a	a	a	a	b	ab	ab	a	b	a
PSG-B	a	ab	a	a	a	ab	ab	ab	a	a	b
PSG-E	b	a	a	a	a	b	ab	ab	a	bc	b
PSG-I	c	ab	a	a	a	b	b	b	a	c	b
SG	c	b	a	a	a	a	a	a	a	c	ab

Figure 4. Mean soil CO₂ efflux rates (µmol m⁻² s⁻¹) measured approximately every 6 weeks between January 10, 2012 to January 20, 2013 in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. Error bars represent ±1 standard error from the mean. Stars indicate sampling dates with significant differences between treatments as determined using repeated measures analysis ($\alpha = 0.1$). The accompanying matrix represents mean separation using Tukey-Kramer HSD where different letters within each treatment date indicate significant differences. Terms with a single asterisks (*) are significant at $\alpha=0.1$ level, double asterisks (**) at $\alpha=0.05$. P represents traditional pine treatments, SG represents flat planted switchgrass, and PSG represents pine intercropped with switchgrass. Additional treatment designations indicate the microtopographical position of the sample location where B represents the bedded row, I represents the interbed space, and E represents the edge where an aboveground transition from switchgrass to pine can be observed.

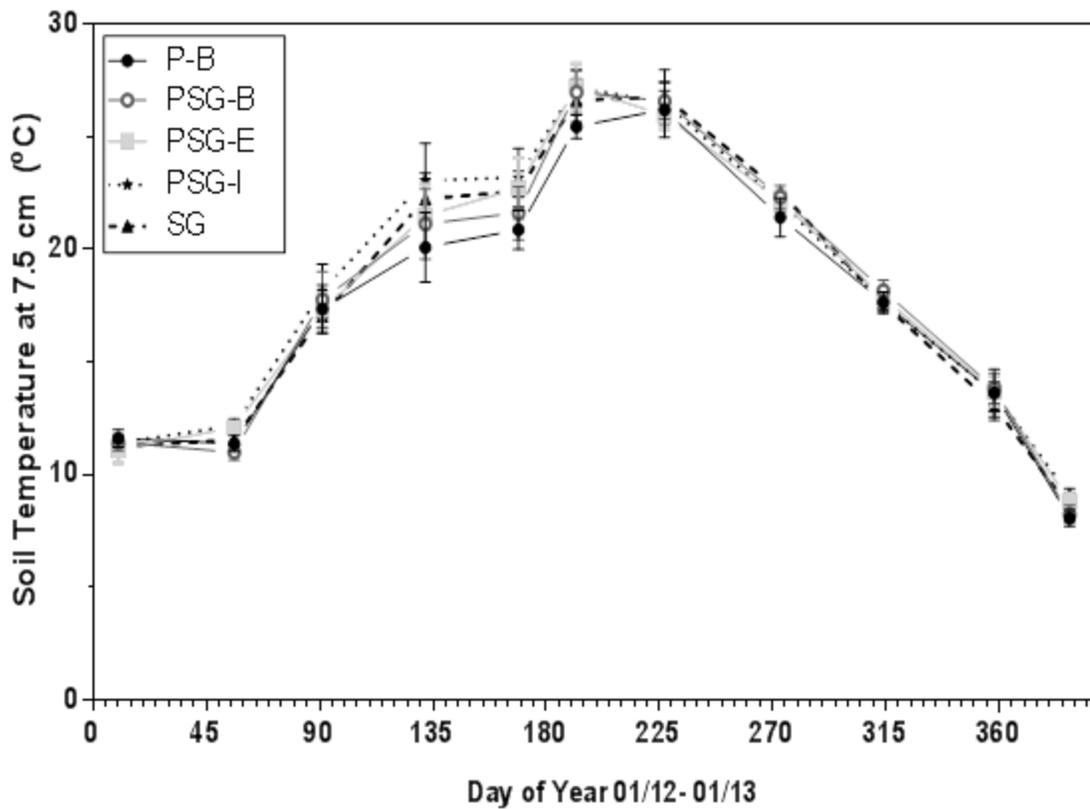
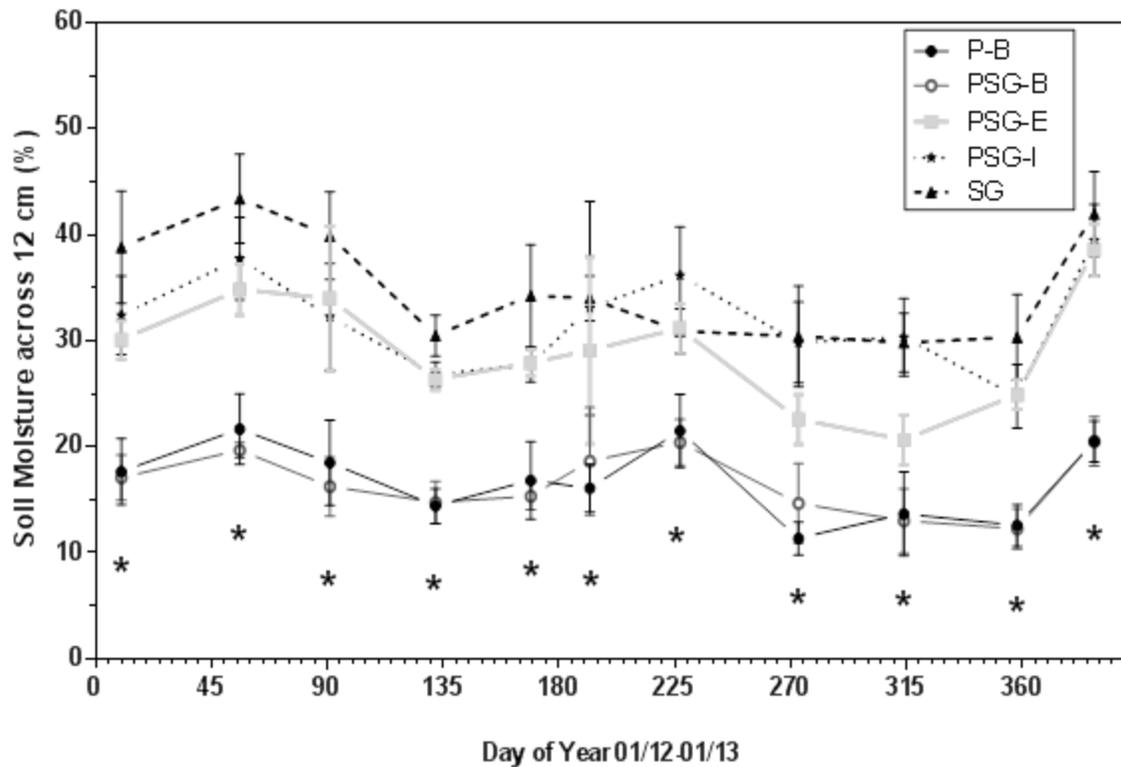


Figure 5. Mean soil temperature (°C at 7.5 cm) measured every 6 weeks between January 10, 2012 to January 20, 2013 in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. Error bars represent ± 1 standard error from the mean. P represents traditional pine treatments, SG represents flat planted switchgrass, and PSG represents pine intercropped with switchgrass. Additional treatment designations indicate the microtopographical position of the sample location where B represents the bedded row, I represents the interbed space, and E represents the edge where an aboveground transition from switchgrass to pine can be observed.



	Jan10	Feb25	Mar30	May10	Jun16	Jul10	Aug14	Sep15	Oct26	Dec09	Jan19
PB	b	b	b	b	b	c	b	b	b	b	b
PSG-B	b	b	b	b	b	bc	b	b	b	b	b
PSG-E	a	a	a	ab	a						
PSG-I	a	a	a	ab	ab	a	a	a	a	ab	a
SG	a	a	a	a	a	a	ab	a	a	a	a

Figure 6. Mean soil moisture (% across 12 cm) measured approximately every 6 weeks between January 10, 2012 to January 20, 2013 in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. Error bars represent ± 1 standard error from the mean. Stars indicate sampling dates with significant differences between treatments as determined using repeated measures analysis ($\alpha = 0.05$). The accompanying matrix represents mean separation using Tukey-Kramer HSD where different letters within each treatment date indicate significant differences at $\alpha = 0.05$ where P represents traditional pine treatments, SG represents flat planted switchgrass, and PSG represents pine intercropped with switchgrass. Additional treatment designations indicate the microtopographical position of the sample location where B represents the bedded row, I represents the interbed space, and E represents the edge where an aboveground transition from switchgrass to pine can be observed.

Collective and individual treatment response of soil CO₂ efflux to temperature change

A significant and positive effect of soil temperature on R_S was noted for all five treatments ($p < 0.0001$) where temperature explained 43% of the variation in the log transformed R_S. Soil moisture and the interaction between soil moisture and temperature did not have a significant effect on R_S.

In order to see if log R_S was responding to temperature similarly in each treatment, we created prediction equation for each of the treatments separately (Fig. 7). Based on the analysis of covariance used to test differences in regression slopes and intercepts (Fig. 7), the regression parameters for SG were significantly different than all other treatments (Table 1). PSG-I also had significantly different intercepts relative to the bedded treatments (P-B and PSG-B).

Table 1: *p*-values representing differences between intercept and slope estimates for the response of log R_S to temperature as influenced by the treatment and microtopographical position in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina.

¹ Treatment	P-B	PSG-B	PSG-E	PSG-I	SG
Differences between slopes					
P-B	-	0.9368	0.1228	0.2251	0.0002
PSG-B		-	0.1322	0.2436	0.0002
PSG-E			-	0.7181	0.0305
PSG-I				-	0.0106
SG					-
Differences between intercepts					
P-B	-	0.6972	0.1278	0.0295	0.00004
PSG-B		-	0.2460	0.0678	0.0001
PSG-E			-	0.5102	0.0085
PSG-I				-	0.0477
SG					-

¹P-B=pine bed; PSG-B=pine+switchgrass bed, PSG-E=pine+switchgrass edge, PSG-I= pine+switchgrass interbed; SG= switchgrass

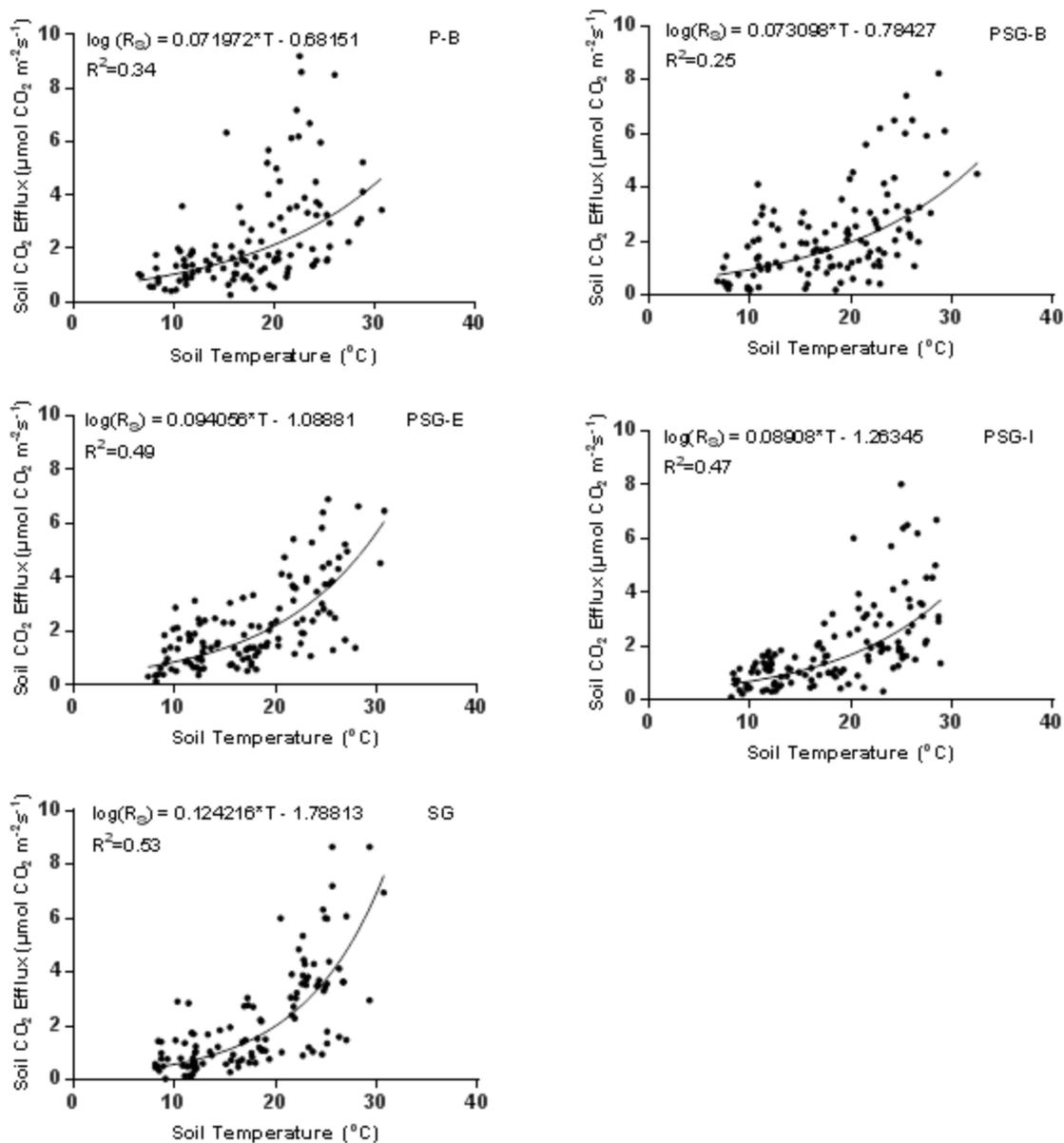


Figure 7. Temperature response of log transformed soil CO₂ efflux measured approximately every 6 weeks between January 10, 2012 to January 20, 2013 in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. P represents traditional pine treatments, SG represents flat planted switchgrass, and PSG represents pine intercropped with switchgrass. Additional treatment designations indicate the microtopographical position of the sample location where B represents the bedded row, I represents the interbed space, and E represents the edge where an aboveground transition from switchgrass to pine can be observed.

Trace gases

Mean N₂O and CH₄ flux did not differ significantly between treatments on any of the sampling periods and the data was highly variable across treatments. Interestingly, both traditional pine (P-B and P-I) treatments were always a source for N₂O whereas the intercropped treatment (PSG-B and PSG-I) exhibited mean values that represent a N₂O sink during some periods (Table 2). Generally, all four treatments were a sink for CH₄ at all times except for the intercrop treatments in the month of August in which positive mean fluxes were reported (Table 2). Pearson's correlation test using all the individual flux measurements showed that both N₂O and CH₄ were significantly and positively correlated with soil temperature but not soil moisture (Table 3).

Table 2: Mean fluxes of nitrous oxide (N₂O) and methane (CH₄) in four different seasons in the bed and interbed of a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. Means are followed by ± 1 standard errors in parenthesis.

N ₂ O-N($\mu\text{g m}^{-2} \text{d}^{-1}$)					
Measurement Date	P-B	P-I	PSG-B	PSG-I	<i>p</i> -value
May 12, 2012	41.38 (8.408)	160.3 (64.69)	96.37 (76.00)	360.8 (331.9)	0.3863
August 14, 2012	141.7 (71.26)	71.47 (61.15)	92.27 (18.10)	65.45 (72.86)	0.5859
November 4, 2012	46.25 (24.70)	72.10 (65.96)	-15.86 (50.62)	-8.820 (14.00)	0.9407
February 2, 2013	41.23 (28.31)	67.71 (23.67)	-25.83 (48.00)	-24.16 (14.05)	0.9137
CH ₄ -C($\mu\text{g m}^{-2} \text{d}^{-1}$)					
May 12, 2012	-126.2 (66.69)	-35.38 (148.6)	-257.9 (36.44)	-122.8 (52.43)	0.906
August 14, 2012	-29.89 (126.5)	-86.16 (110.9)	209.6 (242.3)	23.16 (61.15)	0.6291
November 4, 2012	-217.5 (62.45)	-135.0 (120.0)	-177.2 (59.29)	-222.0 (41.94)	0.3254
February 2, 2013	-222.7 (47.01)	-171.7 (120.8)	-242.1 (84.56)	-253.7 (26.87)	0.277

P-B= pine bed; P-I= pine interbed; PSG-B= pine+switchgrass bed; PSG-I= pine+switchgrass interbed.

Table 3: Pearson’s correlation probability between trace gas fluxes and environmental factors in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina (n=128) combining all the sampling periods; numbers outside parenthesis are correlation and numbers inside parenthesis are *p* values.

	N ₂ O	Soil temperature	Soil moisture
CH ₄	0.2166(0.0141)	0.3418(< 0.0001)	-0.0674(0.4496)
N ₂ O	-	0.2176(0.0136)	-0.0782(0.2779)

Soil CO₂ efflux partitioning

By July 11, 2012 (cores installed in March 30, 2012), measured R_S was lower inside the cores than outside in all treatments (Fig. 8). Based on these data, the estimated proportion of R_S due to R_H was $76 \pm 7\%$, $64 \pm 8\%$ and $67 \pm 2\%$ in PSG-B, PSG-E, and PSG-I (Fig. 9) respectively, with no significant differences in R_H between the three treatments. Thus, averaged across all the treatments, $69 \pm 4\%$ of R_S is attributed to R_H .

In the second set of cores installed in August 14, 2012, R_S rates were very low by the time cores were scheduled to be removed on November 18, 2012. This made partitioning soil respiration difficult due to the low efflux rates in the context of our analytical precision. Thus, for this period of our soil respiration partitioning, we chose to use the previous measurement date [October 26, 2012 (day 73)] for our R_H calculation (Fig. 8). The relative contribution of R_H toward total R_S was estimated to be $91 \pm 25\%$ in PSG-B, $71 \pm 11\%$ in PSG-E and $72 \pm 8\%$ in PSG-I (Fig. 9). No treatment differences were detected, thus the mean proportion of R_H across all the treatments was $78 \pm 7\%$.

Trends in soil moisture inside and outside the cores showed different patterns. There were no observed differences in temperature with the root exclusion cores (Table 4). When soil moisture was taken from directly inside the cores following R_S measurements in May, July and November, moisture content was higher inside the cores relative to outside the cores, with differences being significant for May and July (Table 5).

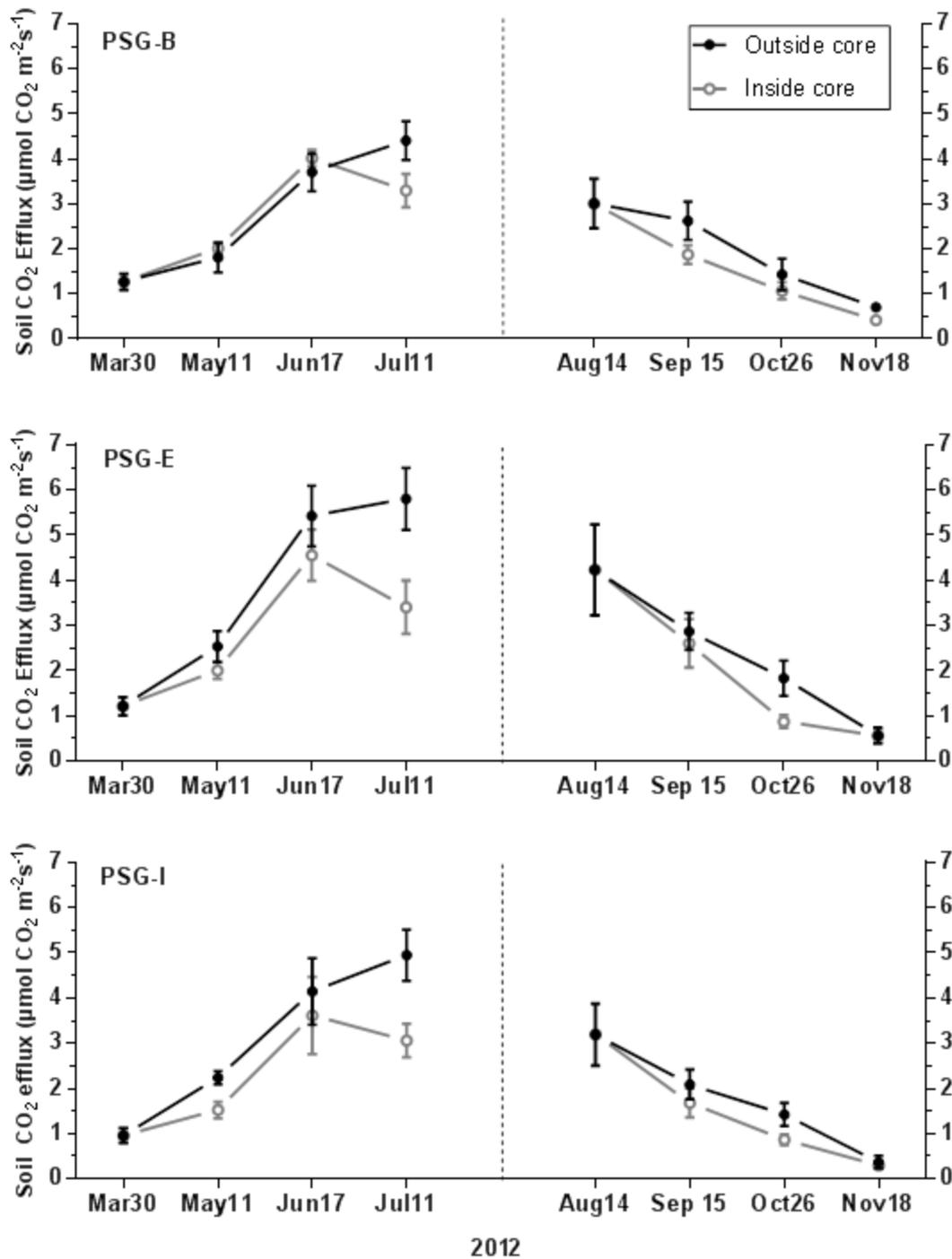


Figure 8. R_S rates measured inside and outside cores in summer (Mar 30- Jul 11) and fall (Aug 14- Nov 18) of 2012 in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. Error bars represent ±1 standard error from the mean. PSG treatment represents pine intercropped with switchgrass. Additional treatment designations indicate the microtopographical position of the sample location where B represents the bedded row, represents the interbed space, and E represents the edge where an aboveground transition from switchgrass to pine can be observed.

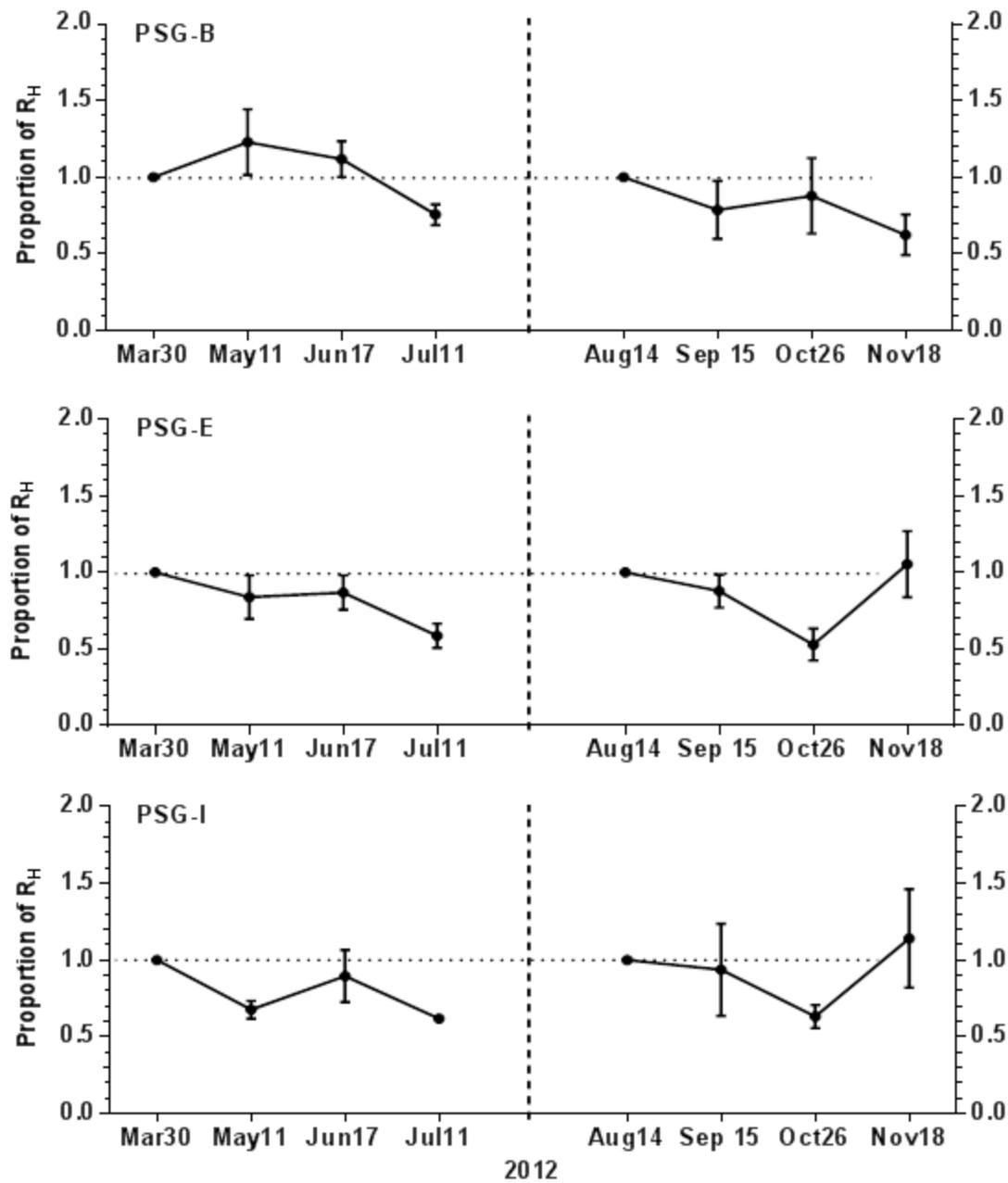


Figure 9. The proportion of R_S attributed to R_H calculated by dividing R_S rates inside cores by R_S rates outside cores in summer (Mar 30- Jul 11) and fall (Aug 14- Nov 18) of 2012 in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. Error bars represent ± 1 standard error from the mean. PSG treatment represents pine intercropped with switchgrass. Additional treatment designations indicate the microtopographical position of the sample location where B represents the bedded row, I represents the interbed space, and E represents the edge where an aboveground transition from switchgrass to pine can be observed.

Table 4: Soil temperature at 7.5 cm soil depth measured inside and outside cores in summer (Mar 30- Jul 11) and fall (Aug 14- Nov 18) of 2012 in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. Soil temperature inside collars was measured in May and July only. Means are followed by ± 1 standard errors in parenthesis.

<u>Measurement</u>	<u>PSG-B</u>		<u>PSG-E</u>		<u>PSG-I</u>	
	<u>Inside core</u>	<u>Outside core</u>	<u>Inside core</u>	<u>Outside core</u>	<u>Inside core</u>	<u>Outside core</u>
May11	21.2 (1.3)	21.1 (1.6)	21.9 (1.4)	21.9 (1.4)	22.9 (1.5)	23.1 (1.6)
Jul11	25.0 (0.4)	25.0 (0.4)	25.2 (0.3)	25.3 (0.3)	25.5 (0.3)	25.6 (0.3)

PSG-B= pine+switchgrass bed, PSG-E= pine+switchgrass edge, PSG-I= pine+switchgrass interbed

Table 5: Volumetric soil moisture content across 0 to 12 cm measured inside and outside cores in summer (Mar 30- Jul 11) and fall (Aug 14- Nov 18) of 2012 in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. Measurements inside cores were done in May, July and November only. Different letters indicate significant differences between inside and outside core moisture within a treatment at $\alpha = 0.05$ using t-test. Means are followed by ± 1 standard errors in parenthesis.

<u>Measurement</u>	<u>PSG-B</u>		<u>PSG-E</u>		<u>PSG-I</u>	
	<u>Inside core</u>	<u>Outside core</u>	<u>Inside core</u>	<u>Outside core</u>	<u>Inside core</u>	<u>Outside core</u>
May11	22.3 ^a (3.2)	14.8 ^b (2.0)	38.9 ^a (3.7)	26.3 ^b (1.0)	35.9 ^a (2.4)	26.8 ^b (1.6)
Jul11	20.8 ^a (3.6)	9.9 ^b (1.2)	35.1 ^a (4.7)	17.6 ^b (3.6)	29.8 ^a (1.9)	25.6 ^b (0.3)
Nov18	22.9 (4.7)	20.0 (4.3)	36.4 (9.0)	30.3 (2.2)	37.8 (3.1)	36.6 (3.7)

PSG-B= pine+switchgrass bed, PSG-E= pine+switchgrass edge, PSG-I= pine+switchgrass interbed

Root biomass, length and surface area

Data from fresh cores in July show that total (0-35 cm) switchgrass root biomass was greatest in SG relative to the other treatments (Table 6). At the surface (0-15cm), switchgrass root biomass declined significantly across the following treatments: SG, PSG-I, PSG-E, and PSG-B (Table 6). At depth (15-35 cm), fewer significant differences in switchgrass root biomass were found, although SG remained highest (Table 6). Trends in switchgrass root length and surface area were similar to those observed in biomass (Table 6).

Total (0-35 cm) pine root biomass was greatest on bedded locations (P-B and PSG-B), irrespective of intercropping, followed by the location immediately adjacent to the beds (PSG-E) (Table 6). Trends within the surface (0-15 cm) depth increment were less clear, although the subsurface (15-35 cm) depth increment seemed to drive the total pine root biomass observations (Table 6). Trends in pine root length and surface area were similar to those observed in biomass (Table 6).

Further data for the fresh cores (i.e., not root exclusion cores) can be found in Appendix A. It is worth noting that the majority (over 90%) of the switchgrass roots in the fresh cores were fine roots (≤ 2 mm in diameter). Similar trend was observed in the roots obtained from root exclusion cores. Similarly, a majority (over 96%) of loblolly pine roots were fine roots. Over 96% of loblolly pine root length comprised of fine roots (≤ 2 mm in diameter). This also matched the observation of roots extracted from root exclusion cores. The proportion of coarse roots contributing to total pine root surface area in the beds (P-B and PSG-B) was greater in 15-35 cm soil depth relative to 0-15 cm soil depth.

Generally, pine roots were more evenly distributed between the depths while switch grass roots were more concentrated in the 0-15 cm depth (Table 6). This pattern can be seen with all

the root variables. Total switchgrass root biomass (5359 g m^{-3}) in the SG treatment exceeded the total pine root biomass value (3262 g m^{-3}) in the pine bed (Table 6).

Comparison of root biomass in the fresh cores versus root exclusion cores collected in July showed 63%, 45% and 49% less root biomass inside root exclusion cores in PSG-B, PSG-E and PSG-I treatments respectively (Appendix C).

Correlations between roots, R_A and R_S

R_A was significantly correlated with switchgrass root biomass and surface area, although not with root length (Table 7). Specifically, total grass root, fine root (1-2 mm) surface area and medium root (2-4 mm) surface area were significantly and positively correlated with R_A , while pine root biomass was significantly and negatively correlated with R_A (Table 7). R_S was significantly and positively correlated with total root biomass only ($r = 0.2938$, $p = 0.0867$; Table 7).

Table 6: July switchgrass and loblolly pine root biomass (g m^{-3}), average root length (cm dm^{-3}), and average root surface area ($\text{cm}^2 \text{dm}^{-3}$) in the fresh cores from 0-15 cm, 15-35 cm soil depth and total depth in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina. Means are followed by ± 1 standard errors in parenthesis. Different letters following the means indicate significant differences across treatments within a depth at $\alpha = 0.05$.

Root Variable	¹ Treatment	Pine			Switchgrass		
		0-15 cm	15-35 cm	Total	0-15 cm	15-35 cm	Total
<u>Biomass</u> (gm^{-3})	P-B	1080 ^a (217.7)	2182 ^a (519.6)	3262 ^a (501.1)	NA	NA	NA
	PSG-B	996.8 ^a (290.2)	2838 ^a (644.4)	3835 ^a (628.7)	116.9 ^c (22.37)	65.94 ^b (30.59)	182.8 ^b (51.36)
	PSG-E	1161 ^a (292.0)	505.2 ^b (176.3)	1666 ^b (197.0)	736.7 ^b (195.1)	510.0 ^{ab} (302.6)	1247 ^b (424.4)
	PSG-I	241.6 ^{ab} (61.59)	84.39 ^b (25.70)	325.9 ^c (56.28)	1308 ^{ab} (405.6)	173.1 ^b (17.37)	1481 ^b (418.7)
	SG	138.5 ^b (95.20)	272.5 ^b (89.09)	411.0 ^c (178.2)	3620 ^a (1358)	1739 ^a (524.6)	5359 ^a (1842)
<u>Length</u> (cm dm^{-3})	P-B	1297 ^a (252.7)	1038 ^a (303.0)	2335 ^a (532.0)	NA	NA	NA
	PSG-B	630.7 ^a (105.5)	414.0 ^{ab} (78.23)	1045 ^b (170.7)	864.59 ^c (238.5)	285.0 ^a (115.6)	1150 ^c (330.1)
	PSG-E	721.2 ^a (93.42)	122.9 ^{bc} (41.29)	844.1 ^{bc} (60.38)	2568 ^b (533.4)	373.0 ^a (132.4)	2941 ^{bc} (565.7)
	PSG-I	258.6 ^{ab} (57.44)	53.41 ^c (9.668)	312.0 ^{cd} (56.65)	4029 ^{ab} (990.7)	473.3 ^a (190.3)	4503 ^{ab} (1163)
	SG	80.07 ^b (67.52)	78.32 ^{bc} (13.33)	158.4 ^d (76.94)	6581 ^a (905.58)	1084 ^a (300.3)	7665 ^a (640.9)
<u>Surface Area</u> ($\text{cm}^2 \text{dm}^{-3}$)	P-B	16.76 ^a (2.619)	19.10 ^a (4.884)	35.76 ^a (7.287)	NA	NA	NA
	PSG-B	10.25 ^{ab} (1.938)	9.252 ^{ab} (1.616)	19.50 ^{ab} (3.457)	7.662 ^c (2.048)	2.808 ^b (0.9621)	10.47 ^c (2.787)
	PSG-E	13.32 ^{ab} (2.392)	2.708 ^{bc} (0.9730)	16.03 ^{bc} (1.712)	26.35 ^{bc} (5.689)	5.150 ^b (1.467)	31.50 ^{bc} (6.147)
	PSG-I	5.035 ^{bc} (1.295)	1.153 ^c (0.1758)	6.188 ^{cd} (1.239)	36.67 ^b (8.667)	5.352 ^b (1.252)	42.02 ^b (9.801)
	SG	1.236 ^c (0.989)	1.664 ^{bc} (0.3909)	2.900 ^d (1.270)	71.53 ^a (4.495)	18.89 ^a (6.992)	90.41 ^a (4.807)

¹P-B=pine bed; PSG-B=pine+switchgrass bed, PSG-E=pine+switchgrass edge, PSG-I= pine+switchgrass interbed; SG= switchgrass

Table 7: Spearman’s correlation constants (r , left) and p -values (in parentheses, right) between root parameters in 0-35 cm soil depth and total R_S ³, and proportion of autotrophic respiration to R_S (R_A)⁴ in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina.

	R_A	R_S
Pine biomass	-0.2408(0.0416)	0.1395(0.4242)
Grass biomass	0.3707(0.0013)	0.0591(0.7359)
Total root biomass	-0.0521(0.6640)	0.2938(0.0867)
Fine root surface area	0.2645(0.0248)	0.1824(0.2944)
Medium root surface area	0.1991(0.0936)	0.0381 (0.8280)
Fine root length	0.0388(0.7463)	0.2515(0.1449)
Medium root length	-0.0913(0.4457)	0.0588(0.7371)
Coarse woody debris	-0.0985(0.4104)	0.0958(0.5841)

³Roots correlated with R_S involve roots collected from fresh cores in PSG-B, PSG-E and PSG-I

⁴Roots correlated with R_A involve roots from exclusion cores in PSG-B, PSG-E and PSG-I

DISCUSSION

Soil CO₂ efflux

Our objective was to determine soil CO₂ efflux from monoculture and co-culture stands of loblolly pine and switchgrass during their fourth growing season. R_S in all treatments was the greatest in the warmer months. R_S in the SG treatment in particular increased dramatically during the growing season (Fig. 4). The peak growing season R_S rates from the SG treatment are in the range of 6 μmol CO₂ m⁻² s⁻¹, while winter rates were as low as 0.8 μmol CO₂ m⁻² s⁻¹ at this site (Fig. 4). Similarly, a study in South Dakota by Lee et al. (2007) reported peak growing season switchgrass R_S ranging from 4-6 μmol CO₂ m⁻² s⁻¹, 5-7 μmol CO₂ m⁻² s⁻¹, and 8-12 μmol CO₂ m⁻² s⁻¹ from control, nitrogen and manure-N applied switchgrass establishments and winter rates of below 1 μmol CO₂ m⁻² s⁻¹. Since R_S is related to overall productivity (Raich and Nadelhoffer 1989, Knoepp et al. 2000) our R_S rates suggests the SG treatment could perform better since rates are only in the mid-range of the Lee et al. (2007) study. Peak growing season R_S from the PSG-I switchgrass is significantly lower (in the range of 4 μmol CO₂ m⁻² s⁻¹; Fig. 4) suggesting its productivity may be impaired.

R_S from this four-year old loblolly pine stands (traditional and co-culture) were within the ranges (1-5 μmol CO₂ m⁻² s⁻¹; Fig. 4) observed for loblolly pines growing in diverse sites. A study by Gough et al. (2005) reported seasonal R_S rates between 1-5 μmol CO₂ m⁻² s⁻¹ in 4-year-old loblolly pine stand in Virginia Piedmont. The same study reported similar seasonal R_S rates for 6-year-old loblolly pines in South Carolina coastal plain to range from 1-6 μmol CO₂ m⁻² s⁻¹. Another study by Wiseman and Seiler (2004) reported maximum R_S of 4 μmol CO₂ m⁻² s⁻¹ in 1 to 2-year-old loblolly pine stands and 7 μmol CO₂ m⁻² s⁻¹ in 20 to 25-year-old loblolly pine stands located in the Virginia Piedmont.

Switchgrass root productivity and related R_S in SG versus PSG-I

There are relatively fewer switchgrass roots in PSG-I than SG treatment (Table 4). The presence of higher total root biomass, length and surface area in SG versus PSG-I (Table 4) in the upper 15 cm of the soil horizon could greatly influence R_S (Maier and Kress 2000). Overall, the root data and related lower R_S rates suggests that even at age four, intercropping of loblolly pine and switchgrass is negatively affecting belowground switchgrass root productivity.

One of the controls on the contribution of R_A and R_H to R_S is the amount and activity of fine roots (Samuelson et al. 2009). Higher R_S and R_H are positively correlated with increased root growth, organic C additions and root exudates (Raich and Schlesinger 1992, Raich and Potter 1995). Fresh core root data taken from PSG-I and SG show significantly lower switchgrass root surface area (both depths) and root biomass (total and lower depth) in the PSG-I treatment (Table 4). There was also a significant correlation between R_A and switchgrass roots present in the upper 35 cm of soil ($r = 0.37$, $p = 0.0013$; Table 5). Therefore, lower amounts of switchgrass roots present in PSG-I likely contribute to the overall lower R_S in this system. Our data showed that although switchgrass roots alone did not significantly correlate with R_S , total root biomass did ($r = 0.2938$, $p = 0.0867$; Table 5), and the fact that majority of the roots in SG and PSG-I plots comprised of switchgrass roots may have influence over R_S from those plots.

Although not measured experimentally in this study, switchgrass was observed to be growing more densely throughout the measurement plots in monoculture where loblolly pines were not present. Albaugh et al. (2012) had measured switchgrass productivity from May to October 2010 on the same treatment plots and had reported significantly taller switchgrass in SG plots relative to PSG-I plots (114 ± 2 cm vs. 98 ± 1 cm respectively) at the end of the growing

season. No significant treatment effect on any of the other measured variables such as percent cover, leaf area index or aboveground biomass had been observed however.

Soil moisture and temperature influence on soil CO₂ efflux

Soil temperature was highly correlated with R_S ($p < 0.001$), as shown by others (Lin et al. 1999, Pang et al. 2013), while soil moisture was not. Unlike soil temperature, which almost always exhibits a positive relationship with R_S, the influence of soil moisture on R_S is equivocal (Maier and Kress 2000, Maier et al. 2004). The poor relationship between soil moisture and R_S in our study could be the result of relatively small seasonal range or variation of soil moisture content during sampling periods (Fig. 6) and more intensive sampling may be necessary to determine how soil moisture interacts with temperature to affect R_S or its components.

Out of all the treatments, SG R_S responded most dramatically to temperature increases. In contrast to our study, Jenkins and Adams (2011) measured R_S on grassland and woodland soils (root free) in a laboratory incubation over seven temperature points (5, 10, 15, 20, 25, 30, 35, and 40°C) and found grassland soils responded less dramatically to increases in soil temperature (slower Q₁₀; increased R_S per 10°C increase in temperature) than woodland soils. Their observations were, however, root free soil and *ex situ*. This suggests that the greater temperature sensitivity of R_S in SG may be due primarily to the R_A component. Higher temperature sensitivity of SG R_S rates suggest that increasing temperature will increase R_S from SG at relatively higher rates compared to other treatments.

Direct temperature responses alone may not be driving the seasonality in R_S, but rather, seasonal variation in belowground C allocation (e.g., roots, root exudates). Belowground C allocation has strong seasonality with greater allocation in summer (Hogberg et al. 2001). Roots

from fresh cores obtained in July showed highest amount of root biomass in SG plots relative to other treatments (Table 4) which could influence temperature sensitivity on R_S and therefore contribute to higher R_S in summer from this treatment.

Partitioning of soil CO₂ efflux

The summer and fall root exclusion core study estimated R_H to be $69 \pm 4\%$ and $78 \pm 7\%$ respectively averaged across all treatments. Studies based on tree girdling experiments in temperate forests have reported higher R_H contributions to R_S as opposed to those conducted in boreal forests (Hogberg et al. 2009). This study used root exclusion cores to sever carbohydrate supply to the roots from the tree stem, which like tree girdling experiments result in root death. Root girdling can result in enhanced decomposition of starved roots and associated mycorrhiza by heterotrophs. There should be increased inputs of labile C in the girdled roots for decomposition due to mortality of fine roots and mycorrhizal fungi (the “assart effect”) following severing, which should contribute to increased heterotrophic activity (Chen et al. 2002, Scott-Denton et al. 2006). The site pertaining to this study consists of highly organic soil (in the Pantego series) as this site also originally existed as a wetland before any land management conversion took place in the 1970s (Albaugh et al. 2012). The abundance of soil organic matter contents in this system could also lead to an increased R_H contribution to R_S as opposed to a system where R_H is limited by soil organic C (Ekbald et al. 2002, Bond-Lamberty et al. 2004). Additionally, since our system is only four years old, the R_A contribution may be expected to increase as the roots continue to develop with stand age.

CONCLUSION

Our study indicates that intercropping switchgrass and loblolly pine has negatively affected switchgrass root productivity in the intercropped plots in this 4-year old agroforestry system. Several reasons for this could be shading from loblolly pines and interspecies competition for water and nutrients. R_s , one of the indices of biological activity, was also significantly lower in the intercrop switchgrass relative to monoculture switchgrass during the active growing season. Estimated annual soil CO_2 efflux¹ for the five treatments from highest to smallest are in the order: PSG-E ($608 \text{ g C m}^{-2} \text{ yr}^{-1}$), P-B ($585 \text{ g C m}^{-2} \text{ yr}^{-1}$), SG ($566 \text{ g C m}^{-2} \text{ yr}^{-1}$), PSG-B ($540 \text{ g C m}^{-2} \text{ yr}^{-1}$), and PSG-I ($461 \text{ g C m}^{-2} \text{ yr}^{-1}$).

The site is not a major source of potent greenhouse gases like CH_4 and N_2O . All the treatments were almost always a sink for CH_4 . Although some treatments were positive source of N_2O , the magnitude of fluxes was much lower compared to fluxes reported from cropping systems (Halverson and Grosso 2011, Molodovskaya et al. 2011).

Overall, the estimated contribution from heterotrophic respiration from the 4-year-old PSG treatment is $73 \pm 5\%$, leaving the estimated autotrophic respiration at $27 \pm 5\%$ indicating that majority of the R_s is currently driven by heterotrophs.

The data presented here is one of the components of a larger study to determine the overall environmental and economic sustainability of this agroforestry system. Despite reduced belowground switchgrass productivity in the intercropped plots, long term monitoring to see if there is additional sequestration of C under switchgrass establishment and whether benefits from feedstock costs are able to overcome the costs incurred in establishing and harvesting switchgrass need to be evaluated for the viability of this intercropped system.

¹ Annual soil CO_2 efflux for each of the treatment was calculated by using averaged daily soil temperatures averaged across treatments and the regression equations outlined in Figure 7. Soil temperature was measured every half-hour continuously for the year 2012 at 10 cm depth.

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Appendix A

Mean coarse (>2 mm diameter) and fine (≤2 mm diameter) root length (cm/core) and surface area (cm²/core) of loblolly pine and switchgrass in 0-15 cm and 15-35 cm soil depth measured in fresh cores in July 2012. Means are followed by ±1 standard errors in parenthesis.

<u>Root variable</u>	<u>¹Treatment</u>	<u>0-15 cm soil depth</u>		<u>15-35 cm soil depth</u>	
		<u>Pine roots</u>	<u>SG roots</u>	<u>Pine roots</u>	<u>SG roots</u>
	PB	1509(296.2)	NA	1566(461.8)	NA
<u>Fine Length</u> (cm/core)	PSG-B	730.1(121.9)	1016(279.4)	611.4(122.9)	339.9(78.32)
	PSG-E	818.5(103.2)	3009(623.7)	183.8(60.91)	569.2(206.6)
	PSG-I	296.8(64.25)	4724(1160)	81.4(15.46)	732.5(299.0)
	SG	90.90(76.15)	7691(1083.8)	116.1(19.50)	1635(378.0)
<u>Coarse Length</u> (cm/core)	PB	19.51(4.118)	NA	63.97(17.17)	NA
	PSG-B	12.92(4.282)	2.655(1.943)	38.86(4.546)	0.7243(0.3522)
	PSG-E	31.17(7.101)	16.59(5.443)	9.258(4.370)	16.61(3.770)
	PSG-I	7.861(4.156)	23.23(7.660)	2.478(1.593)	10.95(1.408)
<u>Fine Surface Area</u> (cm ² /core)	SG	3.423(3.423)	63.06(17.99)	6.944(1.442)	67.7(36.12)
	PB	177.1(32.31)	NA	220.5(61.83)	NA
	PSG-B	103.6(16.81)	86.69(21.97)	95.23(17.68)	36.49(8.363)
	PSG-E	123.5(19.38)	297.3(62.87)	32.4(10.19)	67.45(20.93)
<u>Coarse Surface Area</u> (cm ² /core)	PSG-I	52.81(12.25)	412.9(95.70)	15.81(1.659)	75.53(19.84)
	SG	11.12(8.297)	792.2(66.11)	18.18(3.986)	240.1(79.99)
	PB	20.32(4.890)	NA	77.87(18.27)	NA
	PSG-B	17.15(6.364)	3.571(3.092)	50.11(12.68)	0.5307(0.2564)
<u>Coarse Surface Area</u> (cm ² /core)	PSG-E	33.40(9.785)	13.17(4.242)	10.13(5.563)	13.44(3.271)
	PSG-I	6.510(3.456)	19.11(6.664)	2.311(1.672)	8.535(1.090)
	SG	3.442(3.442)	50.45(15.08)	7.961(2.206)	56.53(30.72)

¹P-B=pine bed; PSG-B=pine+switchgrass bed, PSG-E=pine+switchgrass edge, PSG-I= pine+switchgrass interbed; SG= switchgrass

Appendix B

Inside core switchgrass and loblolly pine root biomass (g m^{-3}), average total root length (cm dm^{-3}), and average total root surface area ($\text{cm}^2 \text{dm}^{-3}$) in intercrop versus pure stands in 0-15 cm and 15-35 cm soil depth measured in July. Means are followed by ± 1 standard errors.

Biomass (g m^{-3})	Pine roots	Switchgrass roots
-----0-15 cm soil depth-----		
PSG-B	439.5 \pm 140.9	27.66 \pm 11.68
PSG-E	597.9 \pm 56.20	393.4 \pm 124.2
PSG-I	182.1 \pm 49.30	549.3 \pm 91.48
-----15-35 cm soil depth-----		
PSG-B	971.7 \pm 390.4	41.86 \pm 26.61
PSG-E	512.9 \pm 195.3	94.26 \pm 36.90
PSG-I	46.16 \pm 16.33	147.1 \pm 34.31
Length (cm dm^{-3})	Pine roots	Switchgrass roots
-----0-15 cm soil depth-----		
PSG-B	364.0 \pm 96.67	160.1 \pm 102.4
PSG-E	552.1 \pm 94.17	1005 \pm 223.8
PSG-I	102.4 \pm 14.00	1033 \pm 334.3
-----15-35 cm soil depth-----		
PSG-B	367.7 \pm 83.53	82.13 \pm 54.53
PSG-E	144.6 \pm 35.60	260.8 \pm 51.23
PSG-I	74.96 \pm 32.90	211.0 \pm 48.03
Surface area ($\text{cm}^2 \text{dm}^{-3}$)	Pine roots	Switchgrass roots
-----0-15 cm soil depth-----		
PSG-B	68.44 \pm 14.09	15.42 \pm 9.121
PSG-E	102.77 \pm 17.44	111.7 \pm 24.00
PSG-I	20.19 \pm 2.392	125.9 \pm 39.66
-----15-35 cm soil depth-----		
PSG-B	100.07 \pm 29.20	8.323 \pm 5.264
PSG-E	33.00 \pm 10.83	39.13 \pm 10.15
PSG-I	16.29 \pm 7.408	29.01 \pm 5.275

PSG-B=pine+switchgrass bed, PSG-E=pine+switchgrass edge, PSG-I= pine+switchgrass interbed

Appendix C

Total root biomass inside deep cores used for estimation of R_H compared to fresh cores obtained immediately adjacent in a four-year-old switchgrass and loblolly pine agroforestry system on the lower coastal plain of North Carolina.

July, Total Root Biomass (g m^{-3})	Inside cores	Outside fresh cores
	-----0-35 cm soil depth-----	
PSG-B	1481 \pm 569.2	4018 \pm 680.1
PSG-E	1598 \pm 412.6	2913 \pm 621.4
PSG-I	924.7 \pm 191.4	1807 \pm 475.0

PSG-B=pine+switchgrass bed, PSG-E=pine+switchgrass edge, PSG-I= pine+switchgrass interbed

Appendix D

November switchgrass and loblolly pine root biomass (g m^{-3}), average total root length (cm dm^{-3}), average total root surface area ($\text{cm}^2 \text{dm}^{-3}$) in 0-35 cm soil depth observed from “inside” pipe. Means are followed by ± 1 standard errors.

Biomass (g m^{-3})	Pine roots	Switchgrass roots
	-----0-35 cm soil depth-----	
PSG-B	1420 \pm 250.0	42.90 \pm 28.81
PSG-E	353.0 \pm 100.2	223.4 \pm 68.77
PSG-I	141.9 \pm 45.08	423.5 \pm 73.21
Length (cm dm^{-3})	Pine roots	Switchgrass roots
	-----0-35 cm soil depth-----	
PSG-B	405.8 \pm 107.4	34.27 \pm 13.53
PSG-E	273.5 \pm 65.27	661.7 \pm 93.68
PSG-I	48.46 \pm 15.77	1652 \pm 634.0
Surface Area ($\text{cm}^2 \text{dm}^{-3}$)	Pine roots	Switchgrass roots
	-----0-35 cm soil depth-----	
PSG-B	94.30 \pm 24.64	3.942 \pm 1.939
PSG-E	49.74 \pm 11.14	69.66 \pm 9.189
PSG-I	10.70 \pm 3.729	164.5 \pm 44.92

PSG-B=pine+switchgrass bed, PSG-E=pine+switchgrass edge, PSG-I= pine+switchgrass interbed