EFFECTS OF THROUGHFALL EXCLUSION AND FERTILIZATION ON SOIL CO_2 EFFLUX AND ITS COMPONENTS IN A LOBLOLLY PINE (*PINUS TAEDA*) PLANTATION

by

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(Under the Direction of ROBERT O. TESKEY)

ABSTRACT

Soil CO_2 efflux (E_S) and total below-ground carbon flux ($TBCF$) are two critical processes that determine the balance of soil carbon in forest ecosystems. Soil CO_2 efflux is composed of root CO_2 efflux (E_R), heterotrophic CO_2 efflux (E_H) and ectomycorrhizal hyphae CO_2 efflux (E_M). However, little is known about how E_S and its components and $TBCF$ respond to decreasing soil moisture and changes in soil fertility. The experimental design was a 2×2 factorial combination of fertilization and precipitation replicated in four blocks. Heterotrophic CO_2 efflux, E_R and E_M were separated using trenched and untrenched plots. Monthly we measured E_S, E_H, soil temperature and soil moisture along with leaf area index (LAI) and litterfall. Fertilization reduced E_S and E_R while the 30% throughfall reduction had no effect on E_H, E_R and E_M. There were correlations between E_S normalized to 15°C (E_{15}) and soil moisture in all treatments except throughfall reduction. Heterotrophic CO_2 efflux normalized at 15 °C (E_{H15}) also increased with increasing soil moisture in control and fertilization treatments. Soil temperature and moisture and their interaction explained more variation in E_S and E_H than in E_R and E_M in the four treatments. In all treatments there was a positive relationship between E_H and LAI. The average annual cumulative E_S in the treatments was 544 g C m^{-2} yr^{-1} (fertilization), 645 g C m^{-2} yr^{-1} (fertilization and throughfall reduction), 665 g Cm^{-2} yr^{-1} (throughfall reduction) and 695 g C m^{-2} yr^{-1} (control). The contribution of E_{H} to E_{S} was from 50 to 58% and the contribution of E_{M} to E_{S} ranged from 10% to 12% in all treatments. Fertilization also had significant effects on annual E_{S}, litterfall, and *TBCF*. Compared to the control, fertilization significantly decreased *TBCF* by 22%. Our results indicate that fertilization plays a critical role in carbon sequestration, increasing aboveground growth and decreasing E_{S} and *TBCF*. The effect of reduced soil moisture on E_{S} and its components was less than that of fertilization, and there were interactions between soil moisture, temperature and *LAI* affecting these fluxes.

INDEX WORDS: Soil CO_2 efflux, Root CO_2 efflux, Heterotrophic CO_2 efflux, Ectomycorrhizal hyphae CO_2 efflux, Total belowground carbon flux, Fertilization, Throughfall reduction, *Pinus taeda*

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CHAPTER 1

INTORDUCTION AND LITERATURE REVIEW

We conducted two studies to determine the responses of soil CO_2 efflux (E_S) and its components and total belowground carbon flux (*TBCF*) to increasing soil fertility and decreasing soil water moisture. The information gathered will help quantify carbon sequestration capacity and carbon sink strength in managed pine plantations in current and future climate regimes. These studies were carried out in a young loblolly pine (*Pinus taeda* L.) plantation. The experimental design was a 2 × 2 factorial combination of fertilization (2 levels: control and fertilization) and precipitation (2 levels: control and the 30% throughfall reduction) replicated in four blocks. The first study examined effects of fertilization and throughfall reduction on E_S and *TBCF*. The aim was to quantify impacts of fertilization and throughfall reduction on annual, growing season and dormant season E_S, and determine the response of *TBCF* to throughfall reduction and fertilization treatments. The second experiment examined the sensitivity of the components of E_S (heterotrophic CO_2 efflux, root CO_2 efflux, and CO_2 efflux from ectomycorrhizal hyphae) to fertilization and throughfall reduction and determine the effects of soil physiological and biological factors on E_S and its component fluxes.

<u>Importance of loblolly pine</u>

As one of the most important commercial tree species in the world, loblolly pine not only provides timber and merchantable volume to the U.S. and the global industrial wood, it also plays a crucial role in the region’s carbon budget (Fox et al., 2007; Noormets et al., 2015).

Loblolly pine covers 13.4 million hectares in the Southern United States and is a significant portion (45%) of the commercial forest land in the region (Zhang et al., 2010). Intensive forest management can greatly increase the profitability of pine plantation in the Southeastern US (Allen et al., 2004) and are regarded as some of the most intensively managed forests in the world (Fox et al., 2007). In general, management (e.g. fertilization, thinning) is the key factor controlling biomass production efficiency (the ratio of photosynthesis used to biomass production, a proxy of carbon use efficiency or the *NPP*/*GPP* ratio) in forest ecosystems (Campioli et al., 2015). Managed temperate forests also tend to exhibit high biomass production efficiency under high nutrient availability (high site quality or fertilization) due to a shift in carbon partitioning from below-ground- to aboveground-biomass (Campioli et al., 2015). Forests with high soil fertility also tend to have higher biomass production efficiency than forests with low fertility due to decreased carbon allocation to root symbionts (Vicca et al., 2012).

Fertilization, one of the most common management practices in modern plantation forestry, has been estimated to have increased productivity in loblolly pine plantations by 17% in the Southeastern United States (Fox et al., 2007). From 1969 to 2004, more than 6 million hectares of loblolly pine plantations were fertilized in the region (Albaugh et al., 2007). Those forests have been estimated to sequester 76 Tg C yr^{-1}, accounting for 23% of the regional greenhouse emissions (Han et al., 2007). Owing to their extent and high productivity, loblolly pine plantations play an important role in the region's carbon sequestration capacity and carbon sink strength. Fertilization has been shown to not only increase aboveground carbon pools (stem, branch and foliage) but also increase carbon storage in coarse roots and tap roots in loblolly pine plantations (Albaugh et al., 1998; Lee and Jose, 2003; Jackson et al., 2009; Will et al., 2006). It has been predicted that precipitation will decline by 10 to 30% in the summer in the Southeastern

US (Christensen et al., 2007). Reduced precipitation may cause a moderate decrease in soil water availability in the future (Kunkel et al., 2015; Seager et al., 2009). Thus, studying the effects of manipulative treatments of fertilization and throughfall exclusion on E_S and its components and *TBCF* can help us understand how nutrient and water availability will affect those carbon fluxes in the future.

Soil CO_2 efflux and its components

Soil CO_2 efflux, or soil respiration, is a critical ecosystem process that regulates carbon cycling in terrestrial ecosystems. In 2008, global E_S was estimated to be 98± 12 Pg C y^{-1} (Bond-Lamberty and Thomson, 2010a), which is approximately an order of magnitude larger than the current annual anthropogenic CO_2 emission from fossil fuel combustion (Boden et al., 2010). Autotrophic CO_2 efflux (E_A) and soil heterotrophic CO_2 efflux (E_H) are two component fluxes that have been measured in many studies (Bond-Lamberty et al., 2004; Subke et al., 2006; Zhou et al., 2014; Liu et al., 2016). The autotrophic component is composed of the CO_2 efflux from roots (E_R) and mycorrhizal fungi (E_M). However, few studies have separated E_A into E_R and E_M. From those studies where E_A has been separated into E_R and E_M, the contribution of E_M to E_S has been reported to be between 8-33% in boreal and temperate forests (Hasselquist et al., 2012; Fahey et al., 2005; Langley et al., 2006; Heinemeyer et al., 2007; Heinemeyer et al., 2011; Vallack et al., 2012; Andrew et al., 2014; Neumann and Matzner, 2014).

Soil heterotrophic CO_2 efflux is derived from the activity of free-living microbes and fauna decomposing dead organic matter. The estimated global E_A and E_H were 40 and 51 Pg C yr^{-1}, respectively (Hashimoto et al., 2015). The heterotrophic component has been estimated to contribute from 40% to 65% of E_S in different forest ecosystems (Hanson et al., 2000; Subke et al., 2006).

Effects of fertilization on soil CO_2 efflux and its components

Fertilization, an important and common forest management practice, has greatly increased productivity of forests in the southeastern United States (Fox et al., 2007). Fertilization usually, but not always, causes a decrease in E_S and its component fluxes in forest ecosystems (Butnor et al., 2003; Jackson et al., 2009; Janssens et al., 2010). A decrease in E_A after fertilization has been attributed to: (1) decreased fine root biomass (Haynes and Gower 1995; Olsson et al., 2005; Phillips and Fahey, 2009; Samuelson et al., 2009; Burton et al., 2012); (2) a shift of production from fine roots to coarse root (Maier and Kress, 2000; Retzlaff et al., 2001); (3) changed fine root tissue chemistry (Drake et al. 2008); (4) decreased mycorrhizal fungi distribution and production (Nilsson and Wallander, 2003;Wallander et al., 2011; Hasselquist et al., 2012; and (5) decreased ectomycorrhizal root tip production (Pritchard et al., 2014).

A decrease in E_H after fertilization has been attributed to: (1) a significant reduction in microbial biomass (Treseder, 2008; Janssens et al., 2010; Zhou et al., 2014); (2) a decline in quantity and quality of root exudates which can stimulate saprotrophic activity and decomposition (priming effect) (Henry et al., 2005; Lagomarsino et al., 2006; Janssens et al., 2010); (3) a change of critical gene expression for microbial decomposition and shifts in the microbial community and decomposing-enzyme (Edwards et al., 2011; Gallo et al., 2004; Zak et al., 2011; Zhou et al., 2014); and (4) a reduction in litter decomposition with nitrogen addition (Janssens et al., 2010; Zhou et al., 2014).

Effects of drought on soil CO_2 efflux and its components

Soil water availability is a critical factor that controls all belowground biological activity and so changes in soil moisture are expected to affect E_S (Wu et al., 2011; Liu et al., 2016). It has been predicted that summertime precipitation will decline by 10 to 30% in the southeastern region in this century (Karl et al., 2009; Christensen et al., 2007). Correlations between soil moisture and E_S and its components have been reported in terrestrial ecosystems (Bond-Lamberty et al., 2010b; Suseela et al., 2012; Yan et al., 2010; Wu et al., 2011; Liu et al., 2016). However, there have been positive, neutral and negative effects of soil moisture on E_S reported in loblolly pine plantations (Maier and Kress, 2000; Gough et al., 2005; Selig et al., 2008; Samuelson et al., 2009; Rifai et al., 2010). There have also been mixed effects of drought on E_S observed in other forest ecosystems (de Dato et al. , 2010; Muhr et al., 2009ab; Sowerby et al., 2008; Borken et al., 2006; Wu et al., 2011; Liu et al., 2016). Differences in drought intensity and the interannual variation in precipitation appear to contribute to the variation observed among different studies (Shi et al., 2014; Wu et al., 2011; Liu et al., 2016). Several possible causes for an effect of soil moisture on E_S are (1) changes in fine root production, root elongation and root exudates (Rustad et al., 2000; Burton et al., 2004; Ford et al., 2012); (2) altering the biomass of soil microorganisms, as well as their activity and composition (Wu et al., 2011; Liu et al., 2016); (3) changes to the physical soil environment that affect gas diffusion rates (Daly et al., 2008); (4) altering rates of aboveground C assimilation (Högberg et al., 2001; Ryan and Law, 2005; Tang et al., 2005); and (5) changes in the temperature sensitivity of E_S (Flanagan and Johnson, 2005; Li et al., 2008 ; McCulley et al., 2007).

With respect to the components of E_S, drought has been shown to affect E_H (Scott-Denton , 2006; Borken et al., 2006; Noormets et al., 2010; Suseela et al., 2012), E_A (Cudlín et al. 2007;

Risk et al., 2012), and E_M (Heinemeyer et al., 2007). However, for loblolly pine plantations studies have only examined the effects on E_S and its components of increasing soil moisture by irrigation (Maier and Kress, 2000; Maier et al., 2004; Samuelson et al., 2009) and little is known about the effects of decreased precipitation.

Effects of fertilization and drought on total belowground carbon flux

Total belowground carbon flux (*TBCF*) includes all the carbon inputs used for root production, respiration, root exudates, herbivory and symbionts (Giardina and Ryan, 2002; Chen et al., 2010; Chen et al., 2014). Much less is known about *TBCF* in forest ecosystems than E_S and its components. In a tropical forest, the combination of an increase in *TBCF* and litterfall accounted for an increase in E_S in response to warming but no change in soil organic carbon storage (Giardina et al., 2014). Fertilization and increased soil moisture have been reported to decrease the *TBCF*/*GPP* ratio due to an increase in *GPP* and shifts in carbon allocation from belowground to aboveground (Giardina et al., 2004; Maier et al., 2004; Litton et al., 2007; Chen et al., 2014). Total belowground carbon flux also decreased with increasing soil moisture in *Populus* stands along an elevation gradient (Fischer et al., 2007). However, irrigation increased *TBCF* in an 8 year-old loblolly pine plantation and in 85 to 95 year-old longleaf pine stands (Maier et al., 2004; Ford et al., 2012). All these experiments involved manipulations that increased soil resources (e.g. fertilization, irrigation). However, very little is known about the effects of decreasing soil resources (e.g. drought) on *TBCF*.

Chapter overview

The effects of throughfall reduction and fertilization on soil CO_2 efflux and total belowground carbon flux are described in Chapter 2. Our objectives in that study were to (1) quantify impacts of fertilization and throughfall reduction on annual, growing season and dormant season E_S, and (2) determine the response of *TBCF* to throughfall reduction and fertilization treatments. Our hypotheses were: (1) fertilization decreases E_S and *TBCF* and (2) throughfall reduction increases *TBCF* but decreases E_S. We found that fertilization, but not throughfall reduction, significantly decreased annual cumulative E_S and *TBCF* during the total 36 month experimental period. The experiment in Chapter 3 was designed to examine the sensitivity of the components of soil CO_2 efflux (E_H, E_R and E_M) to fertilization and throughfall reduction and determine the effects of soil physiological and biological factors on soil CO_2 efflux and its component fluxes. The objectives were to (1) examine how soil temperature, moisture and LAI affected E_S (E_H, E_R and E_M); (2) quantify annual, growing, and dormant season E_S, E_H, E_R and E_M; and (3) determine the effects of fertilization and throughfall reduction on E_S, E_H, E_R and E_M. Our hypotheses were: (1) fertilization decreases E_H; (2) throughfall reduction decreases E_R; and (3) *LAI* is positively related with E_H and E_R. We found that fertilization reduced E_S and E_R while 30% throughfall reduction had no effect on E_H, E_R and E_M. Chapter 4 summarizes the major results of this dissertation.

References

Albaugh, T.J., Allen, H.L. & Fox, T.R. (2007) Historical patterns of forest fertilization in the southeastern United States from 1969 to 2004. *Southern Journal of Applied Forestry*, **31**, 129-137.

Albaugh, T.J., Allen, H.L., Dougherty, P.M., Kress, L.W. & King, J.S. (1998) Leaf area and above-and belowground growth responses of loblolly pine to nutrient and water additions. *Forest Science*, **44**, 317-328.

Allen, A.S. & Schlesinger, W.H. (2004) Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. *Soil Biology and Biochemistry*, **36**, 581-589.

Andrew, C. & Lilleskov, E.A. (2014) Elevated CO_2 and O_3 effects on ectomycorrhizal fungal root tip communities in consideration of a post-agricultural soil nutrient gradient legacy. *Mycorrhiza*, **24**, 581-593.

Boden, T.A., Marland, G. & Andres, R.J. (2010) Global, regional, and national fossil-fuel CO_2 emissions. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tenn., USA **doi**,10.3334/CDIAC/00001_V2010

Bond-Lamberty, B. & Thomson, A. (2010a) Temperature-associated increases in the global soil respiration record. *Nature*, **464**, 579-582.

Bond-Lamberty, B. & Thomson, A. (2010b) A global database of soil respiration data. *Biogeosciences*, **7**, 1915-1926.

Bond-Lamberty, B., Wang, C. & Gower, S.T. (2004) A global relationship between the heterotrophic and autotrophic components of soil respiration? *Global Change Biology*, **10**, 1756-1766.

Borken, W., Savage, K., Davidson, E.A. & Trumbore, S.E. (2006) Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biology*, **12**, 177-193.

Burton, A.J., Pregitzer, K.S., Crawford, J.N., Zogg, G.P. & Zak, D.R. (2004) Simulated chronic NO_3^- deposition reduces soil respiration in northern hardwood forests. *Global Change Biology*, **10**, 1080-1091.

Burton, A.J., Jarvey, J.C., Jarvi, M.P., Zak, D.R. & Pregitzer, K.S. (2012) Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. *Global Change Biology*, **18**, 258-266.

Butnor, J.R., Johnsen, K.H., Oren, R. & Katul, G.G. (2003) Reduction of forest floor respiration by fertilization on both carbon dioxide-enriched and reference 17-year-old loblolly pine stands. *Global Change Biology*, **9**, 849-861.

Campioli, M., Vicca, S., Luyssaert, S., Bilcke, J., Ceschia, E., Chapin III, F., Ciais, P., Fernández-Martínez, M., Malhi, Y. & Obersteiner, M. (2015) Biomass production efficiency controlled by management in temperate and boreal ecosystems. *Nature Geoscience*, **8**, 843-846.

Chen, G., Yang, Y., Guo, J., Xie, J., & Yang, Z. (2010) Relationships between carbon allocation and partitioning of soil respiration across world mature forests. *Plant Ecology*, **212**, 195-206.

Chen, G., Yang, Y. & Robinson, D. (2014) Allometric constraints on, and trade-offs in, belowground carbon allocation and their control of soil respiration across global forest ecosystems. *Global Change Biology*, **20**, 1674-1684.

Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, R., Jones, R., Kolli, R.K., Kwon, W. & Laprise, R. (2007) Regional climate projections. *Climate Change, 2007: The Physical Science Basis. Contribution of Working group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, University Press, Cambridge, Chapter **11***, 847-940.

Cudlín, P., Kieliszewska-Rokicka, B., Rudawska, M., Grebenc, T., Alberton, O., Lehto, T., Bakker, M.R., Børja, I., Konôpka, B. & Leski, T. (2007) Fine roots and ectomycorrhizas as indicators of environmental change. *Plant Biosystems*, **141**, 406-425.

Daly, E., Palmroth, S., Stoy, P., Siqueira, M., Oishi, A.C., Juang, J.-Y., Oren, R., Porporato, A. & Katul, G.G. (2009) The effects of elevated atmospheric CO_2 and nitrogen amendments on subsurface CO_2 production and concentration dynamics in a maturing pine forest. *Biogeochemistry*, **94**, 271-287.

de Dato, G.D., De Angelis, P., Sirca, C. & Beier, C. (2010) Impact of drought and increasing temperatures on soil CO_2 emissions in a Mediterranean shrubland (gariga). *Plant and Soil*, **327**, 153-166.

Drake, J.E., Stoy, P.C., Jackson, R.B. & DeLucia, E.H. (2008) Fine-root respiration in a loblolly pine (Pinus taeda L.) forest exposed to elevated CO_2 and N fertilization. *Plant Cell Environment*, **31**, 1663-72.

Edwards, I.P., Zak, D.R., Kellner, H., Eisenlord, S.D. & Pregitzer, K.S. (2011) Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. *PloS one*, **6**, e20421.

Fahey, T.J., Tierney, G.L., Fitzhugh, R.D., Wilson, G.F. & Siccama, T.G. (2005) Soil respiration and soil carbon balance in a northern hardwood forest ecosystem. *Canadian Journal of Forest Research*, **35**, 244-253.

Fischer, D.G., Hart, S.C., LeRoy, C.J. & Whitham, T.G. (2007) Variation in below-ground carbon fluxes along a *Populus* hybridization gradient. *New Phytologist*, **176**, 415-25.

Flanagan, L.B. & Johnson, B.G. (2005) Interacting effects of temperature, soil moisture and plant biomass production on ecosystem respiration in a northern temperate grassland. *Agricultural and Forest Meteorology*, **130**, 237-253.

Ford, C.R., McGee, J., Scandellari, F., Hobbie, E.A. & Mitchell, R.J. (2012) Long-and short-term precipitation effects on soil CO_2 efflux and total belowground carbon allocation. *Agricultural and Forest Meteorology*, **156**, 54-64.

Fox, T.R., Allen, H.L., Albaugh, T.J., Rubilar, R. & Carlson, C.A. (2007) Tree nutrition and forest fertilization of pine plantations in the southern United States. *Southern Journal of Applied Forestry*, **31**, 5-11.

Gallo, M., Amonette, R., Lauber, C., Sinsabaugh, R. & Zak, D. (2004) Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microbial Ecology*, **48**, 218-229.

Giardina, C.P. & Ryan, M.G. (2002) Total Belowground Carbon Allocation in a Fast-growing Eucalyptus Plantation Estimated Using a Carbon Balance Approach. *Ecosystems*, **5**, 487-499.

Giardina, C.P., Litton, C.M., Crow, S.E. & Asner, G.P. (2014) Warming-related increases in soil CO_2 efflux are explained by increased below-ground carbon flux. *Nature Climate Change*, **4**, 822-827.

Giardina, C.P., Binkley, D., Ryan, M.G., Fownes, J.H. & Senock, R.S. (2004) Belowground carbon cycling in a humid tropical forest decreases with fertilization. *Oecologia*, **139**, 545-50.

Gough, C.M., Seiler, J.R., Wiseman, P.E. & Maier, C.A. (2005) Soil CO_2 efflux in loblolly pine (*Pinus taeda* L.) plantations on the Virginia Piedmont and South Carolina Coastal Plain over a rotation-length chronosequence. *Biogeochemistry*, **73**, 127-147.

Han, F.X., Plodinec, M.J., Su, Y., Monts, D.L. & Li, Z. (2007) Terrestrial carbon pools in southeast and south-central United States. *Climatic Change*, **84**, 191-202.

Hanson, P., Edwards, N., Garten, C. & Andrews, J. (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*, **48**, 115-146.

Haynes, B.E. & Gower, S.T. (1995) Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiology*, **15**, 317-325.

Hashimoto, S., Carvalhais, N., Ito, A., Migliavacca, M., Nishina, K. & Reichstein, M. (2015) Global spatiotemporal distribution of soil respiration modeled using a global database. *Biogeosciences*, **12**, 4121-4132.

Hasselquist, N.J., Metcalfe, D.B. & Högberg, P. (2012) Contrasting effects of low and high nitrogen additions on soil CO_2 flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. *Global Change Biology*, **18**, 3596-3605.

Heinemeyer, A. & McNamara, N.P. (2011) Comparing the closed static versus the closed dynamic chamber flux methodology: Implications for soil respiration studies. *Plant and Soil*, **346**, 145-151.

Heinemeyer, A., Hartley, I.P., Evans, S.P., Carreira De La Fuente, J.A. & Ineson, P. (2007) Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology*, **13**, 1786-1797.

Henry, H.A., Juarez, J.D., Field, C.B. & Vitousek, P.M. (2005) Interactive effects of elevated CO_2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. *Global Change Biology*, **11**, 1808-1815.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M. & Read, D.J. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, **411**, 789-792.

Jackson, R.B., Cook, C.W., Pippen, J.S. & Palmer, S.M. (2009) Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology*, **90**, 3352-3366.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J. & Law, B.E. (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, **3**, 315-322.

Kunkel, K.E. & Frankson, R.M. (2015) Global Land Surface Extremes of Precipitation: Data Limitations and Trends. *Journal of Extreme Events*, 1550004.

Lagomarsino, A., Moscatelli, M., De Angelis, P. & Grego, S. (2006) Labile substrates quality as the main driving force of microbial mineralization activity in a poplar plantation soil under elevated CO_2 and nitrogen fertilization. *Science of the Total Environment*, **372**, 256-265.

Langley A., J., Chapman, S.K. & Hungate, B.A. (2006) Ectomycorrhizal colonization slows root decomposition: the post-mortem fungal legacy. *Ecology Letters*, **9**, 955-959.

Lee, K. H. & Jose, S. (2003) Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. *Forest Ecology and Management*, **185**, 263-273.

Li, H., Yan, J., Yue, X. & Wang, M.. (2008) Significance of soil temperature and moisture for soil respiration in a Chinese mountain area. *Agricultural and Forest Meteorology*, **148**, 490-503.

Litton, C.M., Raich, J.W. & Ryan, M.G. (2007) Carbon allocation in forest ecosystems. *Global Change Biology*, **13**, 2089-2109.

Liu, L., Wang, X., Lajeunesse, M.J., Miao, G., Piao, S., Wan, S., Wu, Y., Wang, Z., Yang, S., Li, P. & Deng, M. (2016) A cross-biome synthesis of soil respiration and its determinants under simulated precipitation changes. *Global Change Biology*, **22**, 1394-405.

Maier, C.A. & Kress, L.W. (2000) Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability. *Canadian Journal of Forest Research*, **30**, 347-359.

Maier, C.A., Albaugh, T.J., Lee Allen, H. & Dougherty, P.M. (2004) Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: the effect of site resources on the stand carbon balance. *Global Change Biology*, **10**, 1335-1350.

McCulley, R.L., Boutton, T.W. & Archer, S.R. (2007) Soil respiration in a subtropical savanna parkland: response to water additions. *Soil Science Society of America Journal*, **71**, 820-828.

Muhr, J. & Borken, W. (2009a) Delayed recovery of soil respiration after wetting of dry soil further reduces C losses from a Norway spruce forest soil. *Journal of Geophysical Research*, **114**

Muhr, J., Borken, W. & Matzner, E. (2009b) Effects of soil frost on soil respiration and its radiocarbon signature in a Norway spruce forest soil. *Global Change Biology*, **15**, 782-793.

Neumann, J. & Matzner, E. (2014) Contribution of newly grown extramatrical ectomycorrhizal mycelium and fine roots to soil respiration in a young Norway spruce site. *Plant and Soil*, **378**, 73-82.

Nilsson, L.O. & Wallander, H. (2003) Production of external mycelium by ectomycorrhizal fungi in a norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist*, **158**, 409-416.

Noormets, A., Gavazzi, M.J., McNulty, S.G., Domec, J.-C., Sun, G.E., King, J.S. & Chen, J. (2010) Response of carbon fluxes to drought in a coastal plain loblolly pine forest. *Global Change Biology*, **16**, 272-287.

Noormets, A., Epron, D., Domec, J.C., McNulty, S.G., Fox, T., Sun, G. & King, J.S. (2015) Effects of forest management on productivity and carbon sequestration: A review and hypothesis. *Forest Ecology and Management*,

Olsson, P., Linder, S., Giesler, R. & Högberg, P. (2005) Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. *Global Change Biology*, **11**, 1745-1753.

Phillips, R.P., Bernhardt, E.S. & Schlesinger, W.H. (2009) Elevated CO_2 increases root exudation from loblolly pine (*Pinus taeda*) seedlings as an N-mediated response. *Tree physiology*, **29**, 1513-1523.

Pritchard, S.G., Taylor, B.N., Cooper, E.R., Beidler, K.V., Strand, A.E., McCormack, M.L. & Zhang, S. (2014) Long-term dynamics of mycorrhizal root tips in a loblolly pine forest grown with free-air CO_2 enrichment and soil N fertilization for 6 years. *Global Change Biology*, **20**, 1313-26.

Retzlaff, W.A., Handest, J.A., O'Malley, D.M., McKeand, S.E. & Topa, M.A. (2001) Whole-tree biomass and carbon allocation of juvenile trees of loblolly pine (*Pinus taeda*): influence of genetics and fertilization. *Canadian Journal of Forest Research*, **31**, 960-970.

Rifai, S.W., Markewitz, D. & Borders, B. (2010) Twenty years of intensive fertilization and competing vegetation suppression in loblolly pine plantations: Impacts on soil C, N, and microbial biomass. *Soil Biology and Biochemistry*, **42**, 713-723.

Risk, D., Nickerson, N., Phillips, C.L., Kellman, L. & Moroni, M. (2012) Drought alters respired $\delta^{13}CO_2$ from autotrophic, but not heterotrophic soil respiration. *Soil Biology and Biochemistry*, **50**, 26-32.

Rustad, L.E., Huntington, T.G. & Boone, R.D. (2000) Controls on soil respiration: implications for climate change. *Biogeochemistry*, **48**, 1-6.

Ryan, M.G. & Law, B.E. (2005) Interpreting, measuring, and modeling soil respiration. *Biogeochemistry*, **73**, 3-27.

Samuelson, L., Mathew, R., Stokes, T., Feng, Y., Aubrey, D. & Coleman, M. (2009) Soil and microbial respiration in a loblolly pine plantation in response to seven years of irrigation and fertilization. *Forest Ecology and Management*, **258**, 2431-2438.

Scott-Denton, L.E., Rosenstiel, T.N. & Monson, R.K. (2006) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biology*, **12**, 205-216.

Seager, R., Tzanova, A. & Nakamura, J. (2009) Drought in the Southeastern United States: Causes, Variability over the Last Millennium, and the Potential for Future Hydroclimate Change. *Journal of Climate*, **22**, 5021-5045.

Selig, M.F., Seiler, J.R. & Tyree, M.C. (2008) Soil carbon and CO_2 efflux as influenced by the thinning of loblolly pine (*Pinus taeda* L.) plantations on the Piedmont of Virginia. *Forest Science*, **54**, 58-66.

Shi, Z., Thomey, M.L., Mowll, W., Litvak, M., Brunsell, N.A., Collins, S.L., Pockman, W.T., Smith, M.D., Knapp, A.K. & Luo, Y. (2014) Differential effects of extreme drought on production and respiration: synthesis and modeling analysis. *Biogeosciences*, **11**, 621-633.

Sowerby, A., Emmett, B.A., Tietema, A. & Beier, C. (2008) Contrasting effects of repeated summer drought on soil carbon efflux in hydric and mesic heathland soils. *Global Change Biology*, **14**, 2388-2404.

Subke, J.A., Inglima, I. & Cotrufo, M.F. (2006) Trends and methodological impacts in soil CO_2 efflux partitioning: A metaanalytical review. *Global Change Biology*, **12**, 921-943.

Suseela, V., Conant, R.T., Wallenstein, M.D. & Dukes, J.S. (2012) Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Global Change Biology*, **18**, 336-348.

Tang, J., Misson, L., Gershenson, A., Cheng, W. & Goldstein, A.H. (2005) Continuous measurements of soil respiration with and without roots in a ponderosa pine plantation in the Sierra Nevada Mountains. *Agricultural and Forest Meteorology*, **132**, 212-227.

Treseder, K.K. (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters*, **11**, 1111-20.

Vallack, H.W., Leronni, V., Metcalfe, D.B., Högberg, P., Ineson, P. & Subke, J.-A. (2012) Application of nitrogen fertilizer to a boreal pine forest has a negative impact on the respiration of ectomycorrhizal hyphae. *Plant and Soil*, **352**, 405-417.

Vicca, S., Luyssaert, S., Penuelas, J., Campioli, M., Chapin, F., Ciais, P., Heinemeyer, A., Högberg, P., Kutsch, W. & Law, B. (2012) Fertile forests produce biomass more efficiently. *Ecology letters*, **15**, 520-526.

Wallander, H., Ekblad, A. & Bergh, J. (2011) Growth and carbon sequestration by ectomycorrhizal fungi in intensively fertilized Norway spruce forests. *Forest Ecology and Management*, **262**, 999-1007.

Wei, W., Weile, C. & Shaopeng, W. (2010) Forest soil respiration and its heterotrophic and autotrophic components: Global patterns and responses to temperature and precipitation. *Soil Biology and Biochemistry*, **42**, 1236-1244.

Will, R.E., Markewitz, D., Hendrick, R.L., Meason, D.F., Crocker, T.R. & Borders, B.E. (2006) Nitrogen and phosphorus dynamics for 13-year-old loblolly pine stands receiving complete competition control and annual N fertilizer. *Forest Ecology and Management*, **227**, 155-168.

Williams, J.W., Jackson, S.T. & Kutzbach, J.E. (2007) Projected distributions of novel and disappearing climates by 2100 AD. *Proceedings of the National Academy of Sciences*, **104**, 5738-5742.

Wu, Z., Dijkstra, P., Koch, G.W., PeÑUelas, J. & Hungate, B.A. (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology*, **17**, 927-942.

Yan, L., Chen, S., Huang, J. & Lin, G. (2010) Differential responses of auto- and heterotrophic soil respiration to water and nitrogen addition in a semiarid temperate steppe. *Global Change Biology*, **16**, 2345-2357.

Zak, D.R., Pregitzer, K.S., Burton, A.J., Edwards, I.P. & Kellner, H. (2011) Microbial responses to a changing environment: implications for the future functioning of terrestrial ecosystems. *Fungal Ecology*, **4**, 386-395.

Zhang, Y., Majumdar, I. & Schelhas, J. (2010) Changes in Woodland Use from Longleaf Pine to Loblolly Pine. *Sustainability*, **2**, 2734-2745.

Zhou, L., Zhou, X., Zhang, B., Lu, M., Luo, Y., Liu, L. & Li, B. (2014) Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. *Global Change Biology*, **20**, 2332-43.

CHAPTER 2

FERTILIZATION, BUT NOT THROUGHFALL REDUCTION, DECREASED SOIL CO_2 EFFLUX AND TOTAL BELOWGROUND CARBON FLUX IN A LOBLOLLY PINE (*PINUS TAEDA*) PLANTATION1[1]

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Abstract

Soil CO_2 efflux (E_S) and total belowground carbon flux (*TBCF*, the sum of metabolic fluxes to support root production, respiration, root exudates, herbivory and symbionts) are two critical processes that determine the balance of soil carbon in forest ecosystems. However, little is known about how E_S and *TBCF* respond to decreasing soil moisture and changes in soil fertility. This was examined in a loblolly pine plantation in Washington, GA from 2012 to 2015. The experimental design was a 2 × 2 factorial combination of fertilization and precipitation replicated in four blocks. We measured E_S and litterfall along with soil temperature and soil moisture. We also estimated aboveground and belowground production in each year. Our objectives were to (1) quantify impacts of fertilization and throughfall reduction on annual, growing season and dormant season E_S, and (2) determine the response of *TBCF* to throughfall reduction and fertilization treatments. Our hypotheses were: (1) fertilization decreases E_S and *TBCF* and (2) throughfall reduction increases *TBCF* but decreases E_S. Fertilization significantly reduced the grand mean of E_S in the total measurement period, mean E_S in the growing season while there was no effect from reduced throughfall. Soil temperature, soil moisture and their interaction explained 51% to 74% of the variation in E_S in all treatments. Apparent Q_{10} of E_S was higher in the non-drought period than the drought period. Soil CO_2 efflux normalized to 15°C (E_{15}) increased with increasing soil moisture to a maximum at approximately 20% volumetric water content and declined after reaching a threshold in all treatments except throughfall reduction. The average contribution of cumulative E_S in the dormant season to annual cumulative E_S ranged from 16% to 19%. The average annual cumulative E_S in the treatments was 544 g C m^{-2} yr^{-1} (fertilization), 645 g C m^{-2} yr^{-1} (fertilization and throughfall reduction), 665 g C m^{-2} yr^{-1} (throughfall reduction) and 695 g C m^{-2} yr^{-1} (control). Compared to the control treatment,

fertilization significantly reduced annual cumulative E_S by 21%. Fertilization also had significant effects on annual E_S, litterfall, and *TBCF*. Compared to the control, fertilization significantly decreased *TBCF* by 22%. There was a negative linear relationship between stem and branch production and *TBCF*.

We conclude that fertilization plays a critical role in carbon sequestration, increasing aboveground growth and decreasing E_S. The temperature sensitivity of E_S depended on soil moisture which indicates we should be cautious in predicting E_S under the future climate regimes.

Introduction

Soil respiration, measured as the efflux of CO_2 from the soil to the atmosphere (E_S), is a critical ecosystem process that plays a major role in the global carbon cycle. Bond-Lamberty and Thomson (2010a) estimated that global E_S in 2008 was 98 ± 12 Pg C yr^{-1}, which is approximately an order of magnitude larger than the current annual anthropogenic CO_2 emission from fossil fuel combustion (Boden *et al.*, 2010). Soil temperature, moisture and soil fertility are three critical abiotic factors that have been shown to influence E_S in numerous field studies in forest ecosystems (Raich and Schlesinger, 1992; Hanson et al., 2000; Subke et al., 2006; Janssens et al., 2010; Wang et al., 2010; Wu et al., 2011; Zhou et al., 2014; Liu et al., 2016). Fertilization, one of the most common management practices in modern plantation forestry, has been estimated to have increased productivity in loblolly pine plantations by 17% in the Southeastern United States (Fox et al., 2007). Fertilization has been observed to significantly increase E_S in some forest ecosystems (Gallardo and Schlesinger, 1994; Tyree et al., 2006). However, in other forests E_S has been shown to decrease after fertilization or nitrogen addition (Janssens et al., 2010; Zhou et al., 2014) or remain unchanged (Maier and Kress, 2000; Pangle and Seiler, 2002; Samuelson et al., 2009; Rifai et al., 2010). Soil resource availability affects E_S by influencing plant growth and productivity, belowground C allocation, fine root growth, litter quantity and quality, microbial enzyme activity, composition of the decomposer community and decomposability of recalcitrant organic matter in soil (Janssens et al., 2010; Lu et al., 2011; Zhou et al., 2014). The magnitude and direction of the response of E_S to fertilization may depend on stand age, biome type, fertilizer amount and length of time after the fertilization event (Liu and Greaver, 2010; Lu et al., 2011; Zhou et al., 2014).

Soil water availability plays a crucial role in all belowground biological activity and altered soil moisture is expected to affect E_S (Wu et al., 2011; Liu et al., 2016). The relationship between soil moisture and E_S and its components has been expressed using different predictive models in a variety of ecosystems (Bond-Lamberty et al., 2010b; Suseela et al., 2012; Yan et al., 2010; Wu et al., 2011; Liu et al., 2016). However, the effect of soil moisture on E_S, particularly in subtropical and temperate forests, has been questioned because some studies have shown a poor relationship between E_S and soil moisture (Samuelson et al., 2009). The southeastern region in the United States is predicted to have decreased precipitation (Williams et al., 2007; Seager et al., 2009) which is likely to affect the soil water content, and may have an impact on E_S. Decreased precipitation has led to reduced E_S in tropical forests, while no reduction was observed in temperate forests (Wu et al., 2011; Liu et al., 2016). There are at least several explanations for an effect of soil moisture on E_S: changes in root production, root elongation and root exudates (Rustad et al., 2000; Burton et al., 2004; Ford et al., 2012); altering the biomass of soil microorganisms, as well as their activity and composition (Wu et al., 2011; Liu et al., 2016); changes to the physical soil environment that affect gas diffusion rates (Daly et al., 2009); altering rates of aboveground C assimilation (Högberg et al., 2001; Ryan and Law, 2005; Tang et al., 2005); and changes in the temperature sensitivity of E_S (Flanagan and Johnson, 2005; Li et al., 2008 ; McCulley et al., 2007).

Efflux of CO_2 from the soil is also related to the total belowground carbon flux (*TBCF*), which is the sum of the carbon inputs supporting root production, respiration, root exudates, herbivory and symbionts (Giardina and Ryan, 2002; Chen et al., 2010; Chen et al., 2014). Giardina et al. (2014) reported that in a tropical forest, warming-related increases in E_S were closely correlated to increases in the labile carbon from *TBCF* and litterfall. The *TBCF*/*GPP* ratio decreased with increased *GPP* caused by fertilization and increased water availability, reflecting changes in carbon allocation above- and below-ground (Giardina et al., 2004; Maier et al., 2004; Litton et al., 2007; Chen et al., 2014). Fischer et al. (2007) found that *TBCF* decreased with increasing soil moisture in *Populus* stands along an elevation and genetic gradient, consistent with the concept that carbon allocation depends on soil resource supply. All the above experiments involved increasing soil resources (e.g. fertilization, irrigation); however, little is known about the effects of decreasing soil resources (e.g. drought) on *TBCF*.

Our study involved manipulative treatments of fertilization and throughfall reduction in a young loblolly pine plantation. The goal of the study was to improve our understanding of how nutrient and water availability affect E_S and *TBCF*. There are more than 13 million hectares of planted loblolly pine (*Pinus taeda* L.) in the southeastern United States (Zhang et al., 2010), accounting for about 45 percent of the commercial forest land in the region. More than 6 million hectares of planted loblolly pine were fertilized in the southeastern region from 1969 to 2004 and the amount of land fertilized annually has been steadily increasing (Albaugh et al., 2007). Moreover, it has been predicted that summertime precipitation will decline by 10 to 30% in the Southeastern region (Williams et al., 2007 ; Christensen et al., 2007). Our objectives were to (1) quantify impacts of fertilization and throughfall reduction on annual, growing season and dormant season E_S, and (2) determine the response of *TBCF* to throughfall reduction and fertilization treatments. Our hypotheses are: (1) fertilization decreases E_S and *TBCF* and (2) throughfall reduction increases *TBCF* but decreases E_S.

Materials and Methods

Site description and experimental design

The study site was located in a loblolly pine plantation in Washington, GA in Taliaferrro County (33º37´32.61´´ N, 82º47´ 56.54´´ W). The mean annual temperature is 16.1ºC and the 30-year average precipitation was 1109 mm (1983-2012) (Samuelson et al., 2014). The soil surface and subsoil texture is clay loam and clay, respectively. The dominant soil series at the site is Lloyd, with a small portion of the site in the Cecil series. The soil pH is 4.8. At the start of the experiment (2012) the stand was 7-years old and stand density was 1373 trees ha^{-1} in the control plots. In the control plots from 2012 to 2014 basal area increased from 11.79 m^{-2} ha^{-1} in to 19.78 m^{-2} ha^{-1} and leaf area index (*LAI*) increased from 1.9 to 3.0 (Table 2.1). During the three years of the study, annual precipitation was 849 mm in 2012 (260 mm below normal), 1413 mm in 2013 (304 mm above normal) and 989 mm in 2014(120 mm below normal).

The experimental design was a 2 × 2 factorial combination of fertilization (2 levels) and throughfall reduction replicated in four blocks for a total of 16 plots. The treatment plots were 1000 m^2 (34.1 m × 28.0 m) and the central measurement plots were 300 m^2 (21.3 m × 14.0 m). The treatments were: control (C), which received no fertilization and no throughfall reduction; fertilization (F), which was a one-time fertilizer application; throughfall reduction (R) which was a 30% reduction in throughfall, and fertilization-throughfall reduction (FR) which was a one-time fertilizer application and a 30% reduction in throughfall. The fertilizer application included 224 kg N ha^{-1}, 28 kg P ha^{-1} (N and P as a mix of urea: 432 kg ha $^{-1}$ and diammonium phosphate: 140 kg ha $^{-1}$), 56 kg K ha^{-1} (potassium chloride) and a micronutrient blend that was applied at a rate of 22.4 kg ha^{-1}. Throughfall reduction was achieved by installing troughs that covered 30% of the ground area. The troughs were installed between tree rows in each plot of R and FR treatments in May, 2012. Wood structures (1.8m wide and 1.3m high) were erected to hold the troughs that were made of two layers of clear U.V. stabilized coextruded polyethylene with embedded high strength cord (Polyscrim 12, Americover Inc., Escondido, CA, USA). Details about the throughfall reduction structure were described in Samuelson et al. (2014). Litterfall that accumulated in the troughs was distributed within the plot

Soil CO_2 efflux measurement

Soil CO_2 efflux measurements were made over a 36 month period beginning in June 2012. We randomly inserted four soil collars, made from PVC pipe (diameter 10 cm, height 5 cm), directly through the litter layer one week before the first measurements and they remained in the same location for the 3-year study period. We measured instantaneous E_S using a soil chamber (LI-6400-09) connected to a portable infrared gas analyzer (LI-6400, Li-Cor, Inc., Lincoln, NE, USA). Soil temperature (T) was measured at 10cm depth with a soil thermocouple probe (6000-09TC Soil Temperature Probe, LICOR Inc., Lincoln, NE, USA) inserted within 5 cm of the measurement collar. Soil moisture (W) was measured at 20 cm depth using a HydroSense™ (Campbell Scientific Inc, Logan, UT and Campbell Scientific Australia, Townsville, QLD, Australia) positioned vertically through the mineral soil within 5 cm of the soil collar immediately following each E_S measurement. We measured E_S monthly from June, 2012 to May, 2015. Continuous plot soil temperature at 10cm depth (107-L Thermistor, Campbell Scientific, Logan, UT, USA) and moisture at 20 cm depth (Wireless Soil Water Reflectometer, CWS655, Campbell Scientific, Logan, Utah, USA) were also measured by a weather station at the site (Bartkowiak et al. 2015).

Stem and branch production, coarse root and fine root production, and annual litterfall

We measured *DBH* (diameter at breast height, 1.37m) and height of all trees (living and dead) in each plot in December from 2011 to 2014. We used loblolly pine-specific allometric equations developed by Gonzalez-Benecke et al. (2014) to estimate the stem, branch, foliage and bark biomass. Aboveground woody biomass production (stem + branch) was assumed to be equal to net increment in woody biomass.

Coarse root biomass was calculated using the function reported in Miller et al. (2006). We did not directly measure fine root biomass in this study. We used regression models based on *DBH* to estimate the average fine root biomass each year. The models were developed from measured fine root (<5mm) biomass and *DBH* of loblolly pine at a plantation in Aiken, South Carolina (personal communication, Doug Aubrey). To estimate total fine root production, we used the fine root turnover rate of loblolly pine roots < 5mm in diameter reported in Matamala et al. (2003) to calculate dead fine root biomass.

We installed 12 round litter traps (area = 0.5 m^2) in each plot. The litter was collected each month from June, 2012 to May 2015. The litter in the traps was collected and oven-dried at 70°C to constant mass every month. We used the carbon content of different tissues of loblolly pine reported in Zhao et al. (2014): 0.478 in litterfall, 0.458 in wood and coarse root, 0.467 in branches and 0.484 in bark, respectively. We assumed coarse root carbon content was as same as wood. We used a fine root carbon content of 0.43 reported in George et al. (2003).

Soil carbon and nitrogen content

We used a soil corer (diameter = 4 cm) to take 8 soil subsamples from two soil depths (between 0 and 10 cm and between 10 and 20 cm) depths, 4 for each depth. We mixed the 4 subsamples together to get a bulk sample for each plot at each depth. Samples were placed in a dry ventilated room until they reached constant mass. After careful removal of roots, all soil samples were sieved through a 2mm sieve to remove gravel > 2 mm diameter. Total carbon and nitrogen content were measured on the soil samples using a CE Elantech NC 2100 Soil Analyzer (CE Elantech, Inc., Lakewood, NJ, USA).

Estimates of total belowground carbon flux (TBCF)

We used the mass balance approach of Giardina and Ryan (2002) to estimate total belowground carbon flux (*TBCF*). Annual *TBCF* was estimated from the annual estimate of E_S, aboveground annual litterfall carbon flux, the change in root carbon content and annual fine and coarse root production. The soil carbon content was considered to be at steady state because we found no significant change in soil carbon content between 2011 and 2013 (Fig S 2.1). We assumed the forest floor litter contribution to *TBCF* was small because: (1) the soil carbon content in the organic horizon was low (Will et al., 2015); (2) the substrate quality of litterfall was low (personal observation); (3) forest floor litter decomposition was low in a similar loblolly pine plantation (Binkley, 2002); (4) the carbon loss from litterfall decomposition was a component of E_S; and (5) some of the carbon in the litter would have become recalcitrant carbon through microbial immobilization.

Data analysis

We assessed potential relationships between E_S, T and W using the multiple regression procedure of SAS (Version 8.2; SAS Inc., Cary, NC, USA). We performed logarithmic transformations of E_S to achieve linearity and homoscedasticity. We used backward elimination to remove insignificant terms ($\alpha > 0.05$). The final form of the regression model was: $\mathrm{Ln}\ (E_S) = \mathrm{a} + \mathrm{b}\ T + c\ W + \mathrm{d}\ T \times W$. The model $\mathrm{Ln}\ (E_S) = \mathrm{a} + \mathrm{b}T$ was used to calculate Q_{10} using the equation $Q_{10} = \mathrm{e}^{10\mathrm{b}}$. Mean E_S, modeled soil CO_2 efflux (E_{SM}) based on regression models, soil CO_2 efflux normalized to 15ºC (E_{15}), Q_{10}, T and W of each treatment were compared with ANCOVA and Tukey's multiple range tests.

Two –way randomized complete block design analysis of variance (ANOVA) was performed for all the measurements using the PROC GLM multiple regression procedure of SAS (Version 8.2; SAS Inc., Cary, NC, USA). The treatment effects of fertilization and throughfall reduction and their interactions on annual E_S, cumulative E_S in growing season and dormant season, litterfall carbon flux, *TBCF*, coarse root production, total soil carbon were analyzed. Each plot was an experimental unit. The treatment was a fixed effect and the measurement year and block were random effects. We used the Palmer Drought Severity Index (*PDSI*, a measure of drought) in Georgia from February, 2012 to May, 2015 (http://www.ncdc.noaa.gov) to determine the monthly level of drought during the study period.

Results

Treatment effects on E_S, T and W

The average soil temperature at 10cm depth varied significantly by date, from 8.2 ºC to 28.2 ºC during the three-year measurement period, but did not differ significantly among the treatments (Fig.2.1a, Table 2.2). Soil moisture (W) differed significantly by measurement date and was significantly lower in R and FR, compared to the C and F treatments (Fig.2.1b, Table 2.2). Over the 36 month study period, F significantly decreased E_S compared to the other treatments (Table 2.2). The C, R and FR treatments had statistically similar E_S.

Fertilization significantly reduced the grand mean of E_S in the total measurement period, mean E_S in the growing season (March to October), and the average E_S in the dormant season in the 1st and 2nd year (Fig. S 2.2). There were no significant interannual differences in any of the treatments across the total measurement period. However, the treatment means of annual E_S were slightly higher but not statistically significant in 2nd year than in either the 1st or 3rd year after treatment. The apparent Q_{10} of E_S ranged from 2.22 to 2.55 in the four treatments (Fig. S 2.2d). The Q_{10} of E_S in the dormant season was higher than in the growing season (2.34 to 4.69 vs from 1.72 to 1.89).

Seasonal patterns of T and W and E_S, E_{SM} and E_{15}

There were strong seasonal patterns in E_S in the four treatments in the three year period of measurement (Fig.2.2a, Table 2.2). This was reflected in E_{SM}, which indicated that the regression models based on temperature and moisture were able to capture the seasonality in E_S (Figure 2.2b). The E_{SM} values fitted well with measured E_S (Fig.S2.3). The R^2 were 0.87 for C, 0.83 for F, 0.73 for FR and 0.83 for R. Modeled E_S was generally underestimated, especially when E_s was high, except in C. When CO_2 efflux was normalized to 15°C, the seasonal variation was largely eliminated, indicating that soil temperature controlled most of the variation in E_S within a treatment (Fig. 2.2c).

Soil temperature, soil moisture and their interaction explained 51% to 74% of the variation in E_S for the total measurement period among the treatments (Table 2.3). The R^2 was higher in the dormant season than in the growing season in the C and R treatments. The R^2 were similar in the dormant and growing seasons in the F and FR treatments. In non-drought periods, soil temperature explained more variation in E_S than in drought periods in C, FR and R treatments, but less in the F treatment (Fig. 2.3). The apparent Q_{10} of E_S was higher in non-drought periods than in drought periods. The E_{15} increased with increasing soil moisture and declined after reaching a threshold in all treatments except R (Fig. 2.4). Soil moisture accounted for more variation in E_{15} in C than in F and FR. However, there was a weak correlation between E_{15} and soil moisture in the R treatment ($p>0.05$).

Annual Cumulative E_S and TBCF

In the growing season, the F treatment had lower cumulative E_S than the C, R and FR treatments (Fig.2.5). Cumulative E_S of F was 583±20 g C m^{-2} compared to 450±15 g C m^{-2} in C. There were also no treatment effects on cumulative E_S in the dormant season. The average percentage contribution of cumulative E_S in the dormant season to annual cumulative E_S ranged from 16% (F) to 19% (FR). The average annual cumulative E_S was 544 g C m^{-2} yr^{-1} for F, 645 g C m^{-2} yr^{-1} for FR and 665 g C m^{-2} yr^{-1} for R and 695 g C m^{-2} yr^{-1} for C (Fig. 2.6). Compared to C, the F treatment significantly reduced annual cumulative E_S, by 21%. There was no relationship between annual cumulative E_S and litterfall or total soil carbon content.

Fertilization had significant effects on annual E_S, litterfall, and *TBCF* (Fig. 2.6; Table 2.4). There was a marginal increase in coarse root production in the F treatment compared to the other treatments ($p = 0.052$). Litterfall increased in the F treatment and decreased in the R treatment. It also had more litterfall than the other treatments. Compared to C, F significantly decreased *TBCF* by 22%. The average annual *TBCF* was 532 g C m^{-2} yr^{-1} for F, 634 g C m^{-2} yr^{-1} for FR, 662 g C m^{-2} yr^{-1} for R and 685 g C m^{-2} yr^{-1} for C. Fertilization increased litterfall and coarse root production significantly in the 2nd year of the study, which was a year in which the site received a high amount of precipitation. There was a significant interannual difference in litterfall and coarse root production. In addition, there was a negative linear relationship between stem and branch production and *TBCF* (Fig. 2.7). There were no relationships between *TBCF* and litterfall and annual E_S.

Discussion

Fertilization effects on E_S and TBCF

Our results support the first hypothesis that fertilization decreases E_S and *TBCF*. The reduction in E_S by fertilization in our study is consistent with similar observations in some coniferous plantations (Table 2.5). There have been various responses of E_S to fertilization in temperate forests, as indicated by the response ratio (the ratio between the natural log of the means of the treatment and control) in Table 2.5 which ranged from -0.329 (negative effect) to 0.133 (positive effect). The magnitude and direction of the effect of fertilization on E_S in different forest ecosystems varies with stand age, biome type, fertilizer amount and experimental duration (Liu and Greaver, 2010; Lu et al., 2011; Zhou et al., 2014). Fertilization has been shown to increase aboveground wood biomass, leaf biomass, and coarse and tap root biomass (Albaugh et al., 1998, 2007, 2008; Will et al., 2006; Miller et al., 2006; McCarthy et al., 2010; Samuelson et al., 2014), but it has also been observed to decease other belowground components, including fine root and microbial biomass, root exudation, autotrophic respiration (E_A) and heterotrophic respiration (E_H) (Haynes and Gower, 1995; Lee and Jose, 2003; Jackson et al., 2009; Samuelson et al., 2009; Janssens et al., 2010; Rifai et al., 2010; Zhou et al., 2014). In our study, the reduction in E_S was related with a decrease in E_A (Yang et al., in preparation). Similarly, in a 31-year-old red pine (*Pinus resinosa*) plantation, a decrease in E_S after fertilization was due to a reduction in E_A, related with decreased fine root biomass (Haynes and Gower, 1995). A decrease in fine root biomass and E_A was also reported in a 7-year-old loblolly pine plantation, however, E_S remained unchanged due to an increase in E_H (Samuelson et al., 2009). Nitrogen fertilization has also been reported to decrease the amount of CO_2 released from ectomycorrhizal fungi hyphae by reducing colonization and causing ectomycorrhizal fungal malfunction (Teste et al., 2012; Hasselquist et al., 2012; Vallack et al., 2012). Since we did not directly measure fine root biomass, fine root lifespan, or ectomycorrhizal fungi production, we cannot be certain of the cause of the reduction in E_A in this study.

Using a meta-analysis, Janssens et al. (2010) reported that nitrogen additions decreased E_S by 17% in temperate forests, mainly from a reduction in E_H. Using the same approach, Zhou et al. (2014) found that there was a significant decrease in E_H caused by nitrogen fertilization in 101 temperate forests. The decrease in E_H after fertilization could be caused my many factors, including a decline in microbial biomass, a reduction in exudation by roots and mycorrhizae, a decline in the production of lignin-degrading enzymes (Treseder, 2008; Lu et al., 2011; Janssens et al., 2010), a shift in microbial community composition (Gallo et al., 2004; Zak et al., 2011), decreased “microbial N mining” (Fontaine et al., 2003; Michel and Matzner, 2003; Craine et al., 2007) and inhibition of litter decomposition (Janssens et al., 2010; Zhou et al., 2014).

Decreased E_S and increased litterfall in fertilized plots may have been the main causes of the reduction in *TBCF* in our study. Fertilization induces a shift in carbon allocation in favor of above-ground tissue production at the expense of root system investment (Litton et al., 2007). A number of studies have reported that the *TBCF*/*GPP* ratio decreased with increasing *GPP* in response to fertilization or increased water availability (Giardina et al., 2004; Maier et al., 2004; Litton et al., 2007; Chen et al., 2014). However, there is mixed evidence regarding the effect of fertilization on fine root production. Declines in fine root production caused by fertilization have been reported in loblolly pine plantations (Albaugh et al., 1998; Will et al., 2006; Pritchard et al., 2008; Jackson et al., 2009). In contrast, small increases, or no change, in fine root production have also been reported in response to fertilization (Coleman, 2007; Lee and Jose, 2003).

In our study, *TBCF* was underestimated because we did not include the carbon input of root exudates. Phillips et al. (2009) showed that under ambient atmospheric CO_2 concentration, annual root exudation was about 23 g C m^{-2} yr^{-1} and fertilization had no effect on it. That represents a very small contribution to *TBCF*, so even if we took into account the reported values of carbon from root exudates, there would likely be no change in the pattern of *TBCF* among the four treatments. Another uncertainty in our calculation of *TBCF* was the estimate of fine root production. However, a very small contribution of fine root net primary production (*NPP*) to total *NPP* (aboveground and belowground) has been reported in loblolly pine plantations of various ages (Drake et al., 2010; Matamala and Schlesinger, 2000). For example, Drake et al. (2010) reported that the fine root production contributed only 2% to total *NPP* in a 14-year-old loblolly pine stand. Matamala and Schlesinger (2000) reported that fine root *NPP* was about 6% of total *NPP* in other loblolly pine plantations. From those results we concluded that fine root production would have had little impact on the estimate of *TBCF* in our study.

Throughfall reduction effects on E_S and TBCF

Drought did not significantly affect E_S and *TBCF* in our study, which was not consistent with our second hypothesis that drought decreases E_S but increases *TBCF*. There have been positive, neutral or negative effects of drought on E_S reported in studies in different forest ecosystems (Table 2.6). In those studies the response ratio ranged from -0.30 to 0.20. However, in most studies in temperate forests, drought had a negative effect on E_S. Whether drought has an effect on E_s may be related to the intensity of the drought and the interannual variation in precipitation. Drought has been shown to decrease E_H (Scott-Denton, 2006; Borken et al., 2006; Noormets et al., 2010; Suseela et al., 2012), decrease E_A (Risk et al., 2012), decrease both E_A and E_H to the same extent (Schindlbacher et al. 2012) and reduce CO_2 derived from ectomycorrhizal fungi (Heinemeyer et al., 2007). Shi et al. (2014) synthesized data from 39 study sites and found that extreme drought (annual precipitation reduction > 40%) decreased E_H. Prolonged droughts also reduced fine root production (Pritchard et al., 2008) and E_H (Drake et al., 2012). In other severe drought studies, a reduction in E_H has been reported, caused by low soil moisture decreasing substrate availability, suppressing decomposer microbial activity and limiting extracellular enzymes (Borken et al., 2006; Suseela et al., 2012). However, Kerhoulas et al. (2013) found there was no effect on ecosystem respiration in moderate drought (25% to 40% decrease in annual precipitation), which they attributed to roots utilizing deep soil water.

The 30% throughfall reduction in our study created only a moderate degree of drought. At the same site, Samuelson et al. (2014) reported that there was only a small decrease in aboveground growth and leaf gas exchange rates in response to the throughfall reduction treatment. Interannual variation in rainfall contributed to the small effect of the throughfall reduction treatment on growth and gas exchange. Precipitation was 11% below normal, and 27% above normal, in the first and second years of the study, respectively. In the third year of the study, precipitation was 23% below normal, but that followed the extremely wet year, which likely buffered any effects on growth and gas exchange (including E_S) during the following drier year.

Although there was no effect of the throughfall reduction treatment on E_S, we still observed relationships between soil moisture and E_S. Our study showed that E_{15} increased with increasing soil moisture to a broad maximum plateau between about 15 to 25% volumetric water content and then declined. Rifai et al. (2010) also reported that soil moisture affected Es in a loblolly pine plantation. Maier and Kress (2000) observed that there was a positive linear relationship between E_S and volumetric water content when the volumetric water content was between 2 and 10%. Davidson (1998) also found a bimodal pattern between soil moisture and E_S in a temperate forest. Suseela et al. (2012) reported that E_H decreased after volumetric soil water content reached a threshold of 26%. High soil moisture may reduce the diffusion of oxygen and impede microbial activity (Janssens et al., 2010). Low soil moisture may directly affect cell water status and metabolism. However, little or no effect of soil moisture on E_S was reported in two previous studies in loblolly pine plantations (Maier and Kress, 2000; Samuelson et al., 2009). The authors of both studies suggested that the apparent lack of an effect of soil moisture on E_S was due to soil moisture not falling below a critical level that would affect E_A and E_H.

Although Q_{10} of E_S has been considered a fixed value in some ecosystem models, soil moisture and substrate availability can affect the apparent Q_{10} of E_S (Davidson and Janssens, 2006). Although E_S was not significantly affected by the R treatment, the apparent Q_{10} of E_S was lower in that treatment compared to the C treatment during drought periods. Similarly, Davidson et al. (1998) found that E_S had a lower Q_{10} in well-drained sites and a higher Q_{10} in wet sites in temperate mixed hardwood forests. Wang and Yang (2006) observed that the apparent Q_{10} of E_S changed with soil water content in six temperate forests. A reduction in rainfall of 50% also decreased the apparent Q_{10} of E_S in an old-field ecosystem (Suseela et al., 2012; Suseela and Dukes, 2013).

Our results showed there was a negative correlation between aboveground *NPP* and *TBCF*. The negative correlation in this study between aboveground *NPP* and *TBCF* was consistent with results in other forests (McDowell et al., 2001; Reich and Bolstad , 2001; Rodeghiero and Cescatti, 2005; Palmroth et al., 2006; Vogel et al., 2008) but was contrary to the positive relationships reported by Raich and Nadelhoffer (1989), Giardina et al. (2005) and Litton et al. (2007). The relationship between *TBCF* and aboveground *NPP* in a specific forest may depend on forest type and the pattern of carbon allocation in response to soil resource availability. We also observed no effect of a 30% reduction in throughfall on *TBCF*. This treatment only caused a slight, and not statistically significant, decrease in E_S and litterfall carbon flux, and coarse root production did not change. Joslin et al. (2000) found that a 33% reduction in throughfall had no effect on fine root production in a mixed hardwood forest since the low root production during dry periods was compensated by high root growth during wet periods. However, high soil water availability can also increase *TCBF*. Maier et al. (2004) showed that irrigation (to > 40% volumetric soil moisture) increased *TBCF* by more than 30% in an 8 year-old loblolly pine plantation. Ford et al. (2012) found that the *TBCF* increased almost two-fold due to irrigation (an increase of > 50% of annual precipitation) in 85 to 95 year-old longleaf pine stands.

Conclusions

Fertilization, but not a 30% reduction in throughfall, decreased E_S and *TBCF*. The response to fertilization was likely due to a shift of carbon allocation which favored aboveground over belowground carbon allocation. Soil temperature controlled most of the variation in E_S. Monthly changes in soil moisture also affected E_S but to a lesser degree than soil temperature. Our observation that the temperature sensitivity of E_S depended on soil moisture indicates we should be cautious when predicting how E_S will change under future climate regimes. In order to robustly predict the response of E_S to drought, we need more long-term studies with different levels of drought to more comprehensively assess the impacts of soil water availability on belowground carbon components.

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Table 2.1. Site mean annual temperature (MAT) and mean annual precipitation (MAP) as well as mean growth characteristics including leaf area index (LAI) and diameter at breast height (DBH) in control plots from 2011 to 2014.

Year	Age (year)	MAT (ºC)	MAP (mm)	Stand density (tree ha^{-1})	Basal Area (m^{-2}ha^{-1})	DBH (cm)	LAI (m^{2}m^{-2})	Height (m)
2011	6	17.5	939	1383	8.80	8.85		6.3
2012	7	19.8	849	1373	11.79	10.38	1.9	7.2
2013	8	18.2	1413	1373	16.13	12.12	2.7	8.8
2014	9	17.9	989	1373	19.78	13.44	3.0	10.1

Table 2.2. Overall means of soil CO_2 efflux (E_S), modeled soil CO_2 efflux (E_{SM}), soil CO_2 efflux normalized to 15^{o}C (E_{15}), apparent temperature sensitivity (Q_{10}), soil temperature at 10cm soil depth (T) and volumetric water content at 20cm depth (W) during the three-year measurement period (Total), in the growing season (GS) and dormant season (DS). The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

Period	Treatments	E_S	E_{SM}	E_{15}	Q_{10}	T	W
			(µmol CO_2 m^{-2} s^{-1})			(^{o}C)	(%)
Total	C	2.31a (0.10)	2.71 a (0.35)	1.61 a (0.13)	2.39 (0.15)	18.6 (0.20)	23a (1.43)
	F	1.75b (0.06)	1.65b (0.15)	1.24b (0.09)	2.49 (0.19)	18.2 (0.19)	19a (1.11)
	FR	2.07ab (0.09)	1.66 b(0.15)	1.54 a(0.15)	2.22 (0.16)	18.1 (0.16)	17 b(1.18)
	R	2.20a (0.08)	1.95 ab(0.29)	1.53a (0.13)	2.55 (0.16)	18.3 (0.20)	17 b(1.42)
GS	C	2.72a (0.11)	3.17 a(0.21)	1.66a (0.14)	1.81 (0.16)	21.0 (0.21)	20 (1.43)
	F	2.02b (0.07)	1.82 b(0.14)	1.27 b(0.08)	1.76 (0.19)	20.2 (0.21)	19 (1.13)
	FR	2.36ab (0.10)	1.83b (0.14)	1.57a (0.14)	1.72 (0.16)	19.9 (0.17)	17 (1.15)
	R	2.56a(0.10)	2.26 b (0.17)	1.58 a(0.14)	1.89 (0.27)	19.8 (0.20)	17 (1.42)
DS	C	1.07a (0.04)	1.25 (0.11)	1.45 a(0.12)	2.85 bc (0.45)	10.5 (0.17)	25a (1.46)
	F	0.97a (0.03)	1.10 (0.09)	1.17 b(0.10)	3.20 b (0.95)	11.5 (0.14)	20ab (1.03)
	FR	1.20a (0.07)	1.14 (0.10)	1.45 a (0.18)	2.34 a (0.42)	12.3 (0.11)	17b (1.28)
	R	1.14 a(0.03)	0.97 (0.16)	1.37 a (0.10)	4.69 a (0.90)	13.1 (0.20)	16b (1.45)

Table 2.3. Regression models of soil CO_2 efflux (E_S) (μmol CO_2 m^{-2} s^{-1}) predicted using soil temperature at 10cm soil depth (T) and soil volumetric water content at 20cm depth (W). Total represents all measurements made in the 36 month study period. Dormant season (DS) is from November to February in the following year. Growing season (GS) is from March to October. The regression models are of form: Ln (E_S) = a + bT + c W+ d $T \times W$, where Ln is natural logarithm. The lowercase letters a, b, c and d are regression coefficients. The letter n is sample size. Adj-R^2 is the adjusted determination coefficient. RMSE is root mean square error. AICc is the Akaike Information Criterion corrected for finite sample size to account for overfitting with a small sample size. The p-value is the significance level associated with the F-statistic. The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

Period	Treatments	a	b	c	d	n	F	Adj-R^2	RMSE	p	AICc
Total	C	0.234	0.037	-0.037	0.002	108	97.731	0.723	0.300	0.012	52.095
	F	-0.417	0.049			108	60.677	0.617	0.353	0.009	88.222
	FR	-0.387	0.048			108	39.360	0.509	0.396	0.020	114.143
	R	0.292	0.022	-0.059	0.003	108	104.487	0.737	0.291	0.016	45.375
DS	C	0.492	0.011	-0.045		37	15.261	0.517	0.305	0.002	22.896
	F	1.763	-0.136	-0.122	0.010	37	6.361	0.287	0.385	0.010	41.825
	FR	1.473	-0.103	-0.106	0.009	37	2.901	0.125	0.443	0.009	53.436
	R	0.989	-0.027	-0.081		37	14.955	0.511	0.279	0.004	15.526
GS	C	0.989	-0.027			67	11.940	0.319	0.284	0.002	26.816
	F	0.904	0.011			67	13.562	0.350	0.304	0.001	36.128
	FR	0.037	0.034			67	8.240	0.237	0.336	0.003	50.538
	R	0.968	-0.006			67	15.500	0.383	0.286	0.002	27.636

Table 2.4. The *p*-values of treatment effects on soil CO_2 efflux (E_S), carbon flux from litterfall (Litterfall), coarse root production, change in total soil carbon (*TC*), and total belowground carbon flux (*TBCF*) for the total measurement period. The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only. A non-significant treatment effect is indicated by ns.

Treatment	Annual E_S	Litterfall	Coarse root production	*TC*	*TBCF*
F	<.001	<.001	0.052	ns	0.043
R	ns	<.001	ns	ns	ns
FR	ns	ns	ns	ns	ns
Year	ns	0.02	0.0012	ns	0.032
F × Year	ns	0.04	ns	ns	ns
R × Year	ns	0.067	ns	ns	ns
FR × Year	ns	ns	ns	ns	ns

Table 2.5. The response of E_S to fertilization in temperate forest ecosystems. The magnitude of the response was quantified by calculating the response ratio (*RR*) which is the ratio between the natural log of the mean of the treatment (t) and the natural log of the mean of the control (c) = ln ($\overline{Xt}/\ \overline{Xc}$) = ln ($\overline{Xt}$)- ln ($\overline{Xc}$). In the Leaf habit column, E represents evergreen, D represents deciduous, and M represents mixed. All the CO_2 efflux measurements were made with an Infrared Gas Analyzer. Only studies that reported annual E_S were included in the table.

References	Manipulation	Manipulation level	Age	Species	Leaf habit	MAT (°C)	MAP (mm)	Annual *E*s ($gCm^{-2}yr^{-1}$)	*RR*
Maier et al. (2000)	None		11	*Pinus taeda*	E	17	1210	1263	0.023
	Fertilization	Complete nutrition	11	*Pinus taeda*	E	17	1210	1293	
Burton et al. (2004)	None		94	*Acer saccharum*	D	4.8	821	1071	-0.181
	simulated atmospheric NO_3^- deposition	3 g N m^2 yr^{-1}	94	*Acer saccharum*	D	4.8	821	894	
Maier et al. (2004)	None		12	*Pinus palustris*	E	17	1210	811	0.133
	Fertilization	Complete nutrition	12	*Pinus palustris*	E	17	1210	926	
Egli et al. (2001)	None	7 kg N ha^{-1} yr^{-1}	2	*Fagus sylvatica; Picea abies*	M			795	-0.101
	Wet nitrogen deposition	70 kg N ha^{-1} yr^{-1}	2	*Fagus sylvatica; Picea abies*	M			719	
	None	7 kg N-ha^{-1} yr^{-1}	2	*Fagus sylvatica; Picea abies*	M			946	-0.174

	Fertilization	70 kg N ha^{-1} yr^{-1}	2	*Fagus sylvatica; Picea abies*	M			795	
Pangle et al. (2002)	Herbicide		2	*Pinus taeda*	E		1067	422	0
	Herbicide, Fertilization	115 kg ha^{-1} N yr^{-1} and 11.5 kg P -ha^{-1} yr^{-1}	2	*Pinus taeda*	E		1067	422	
Jia et al. (2007)	None			*Fraxinus mandshurica*	D	2.8	723	1173	-0.256
	Fertilization	10 g N- m^{2} yr^{-1}		*Fraxinus mandshurica*	D	2.8	723	908	
	None			*Larix gmelinii*	D	2.8	723	946	-0.329
	Fertilization	10 g N- m^{2} yr^{-1}		*Larix gmelinii*	D	2.8	723	681	
Kim et al. (2008)	Fertilization	112 kg N ha^{-1} year^{-1}, 75 kg P ha^{-1} year^{-1}, 37 kg K ha^{-1} year^{-1}	20	*Larix leptolepis*	D	12.8	1322	908	-0.052
	None		20	*Larix leptolepis*	D	12.8	1322	956	
Samuelson et al. (2009)	None		7	*Pinus taeda*	E	20	1190	1070	-0.195
	Fertilization	Complete nutrition	8	*Pinus taeda*	E	20	1190	880	
	None		7	*Pinus taeda*	E	20	1190	1000	-0.051
	Fertilization	Complete nutrition	8	*Pinus taeda*	E	20	1190	950	
	None		7	*Pinus taeda*	E	20	1190	930	0.092
	Fertilization	Complete nutrition	8	*Pinus taeda*	E	20	1190	1020	
Jassal et al. (2010)	None		58	*Pseudotsuga menziesii*	E	8.6	1450	869	0.066
	Fertilization	200 kg N ha^{-1}yr^{-1}	58	*Pseudotsuga menziesii*	E	8.6	1450	928	
	None		58	*Pseudotsuga menziesii*	E	8.6	1450	836	-0.012
	Fertilization	200 kg N ha^{-1} yr^{-1}	58	*Pseudotsuga menziesii*	E	8.6	1450	826	

Oishi et al. (2014)	None			*Pinus taeda*	E			1268	-0.244
	Fertilization	11.2 g N m^2 yr^{-1}		*Pinus taeda*	E			993	
This study	None		6-8	*Pinus taeda*	E	18.1	1109	695	-0.245
	Fertilization	Complete nutrition	6-8	*Pinus taeda*	E	18.1	1109	544	

Table. 2.6. The response of E_S to drought in temperate forest ecosystems. The magnitude of the response was quantified by calculating the response ratio (*RR*) which is the ratio between the natural log of the mean of the treatment (t) and the natural log of the mean of the control (c) = ln ($\overline{Xt}/\ \overline{Xc}$) = ln ($\overline{Xt}$)- ln ($\overline{Xc}$). Apreci (mm) is the actual annual precipitation in the measurement year in each study. In the Biome column, Tr represents tropical, Te represents temperate, Me represents Mediterranean. In the Leaf habit column, E represents evergreen, D represents deciduous. All the CO_2 efflux measurements were made with an Infrared Gas Analyzer.

References	Manipulation	Age (year)	Species	Biome	Leaf habit	MAT (°C)	MAP (mm)	Apreci (mm)	*Annual E*s ($gCm^{-2}yr^{-1}$)	*RR*
Sotta et al. (2007)	None		Mixed	Tr	E	26	2272		1627	-0.3
	50% rainfall exclusion		Mixed	Tr	E	26	2272		1211	
Borken et al. (2006)	None	60	*Acer rubrum; Quercus rubra*	Te	D	8.5	1050	1095	1148	-0.17
	36-59% growing season precipitation	60	*Acer rubrum; Quercus rubra*	Te	D	8.5	1050	1095	964	
Sowerby et al. (2008)	None		*Calluna vulgaris; Deschampsia flexuosa*	Te	D	9.4		720	597	-0.92
	20-26% reduction in annual precipitation		*Calluna vulgaris; Deschampsia flexuosa*	Te	D	9.4		533	237	
Davidson et al. (2008)	None		Mixed	Tr	E		2000		1280	0
	30-40% annual precip		Mixed	Tr	E		2000		1280	

Metcalfe et al. (2010)	None		Mixed	Tr	E		2500		1300	0
	50% incident rainfall		Mixed	Tr	E		2500		1300	
Brando et al. (2008)	None		Mixed	Tr	E	28	2000		1280	0
	65% annual precipitation		Mixed	Tr	E	28	2000		1280	
Muhr et al. (2009a)	None	145	*Picea abies*	Te	E	5.3	1160		620	-0.2
	Snow removal	145	*Picea abies*	Te	E	5.3	1160		510	
	None	145	*Picea abies*	Te	E	5.3	1160	868	670	-0.16
Muhr et al. (2009b)	100% throughfall exclusion 2 months	145	*Picea abies*	Te	E	5.3	1160	868	570	
	None	145	*Picea abies*	Te	E	5.3	1160	1152	700	-0.17
	100% throughfall exclusion 2 months	145	*Picea abies*	Te	E	5.3	1160	1152	590	
Misson et al. (2010)	None	63	*Quercus ilex*	Te	E	13.5	908		758	-0.11
	100% throughfall exclusion for 6 months	63	*Quercus ilex*	Te	E	13.5	908		677	
	None	63	*Quercus ilex*	Te	E	13.5	908		800	-0.12
	100% throughfall exclusion for 6 months	63	*Quercus ilex*	Te	E	13.5	908		710	

van Straaten et al. (2010)	None	7	*Theobroma cacao; Gliricidia sepium*	Tr	E	25.5	2092		1110	-0.06
	60-80% througfall exclusion	7	*Theobroma cacao; Gliricidia sepium*	Tr	E	25.5	2092		1050	
Cleveland et al. (2010)	None		Mixed	Tr	E	26.5	5000		1142	
	25% reduction in throughfall		Mixed	Tr	E	26.5	5000		1293	0.12
	50% reduction in throughfall		Mixed	Tr	E	26.5	5000		1421	0.09
De Dato et al. (2010)	None	10	*Cistus monspeliensis; Helichrysum italicum; Dorycnium pentaphyllum*	Te	D	16.8	640	598	1173	-0.09
	Spring and autumn drought	10	*Cistus monspeliensis; Helichrysum italicum; Dorycnium pentaphyllum*	Te	D	16.8	640	598	1077	
	None	11	*Cistusmonspeliensis; Helichrysum italicum; Dorycnium pentaphyllum*	Te	D	16.8	640	411	1173	-0.07
	Spring and autumn drought	11	*Cistus monspeliensis; Helichrysum italicum; Dorycnium pentaphyllum*	Te	D	16.8	640	411	1091	
	None	12	*Cistus monspeliensis; Helichrysum italicum; Dorycnium pentaphyllum*	Te	D	16.8	640	528	1009	-0.07
	Spring and autumn drought	12	*Cistus monspeliensis; Helichrysum italicum;*	Te	D	16.8	640	528	941	

		Dorycnium pentaphyllum							
Cotrufo et al. (2011)	None	*Arbutus unedo; Erica arborea; Fraxinus ornus*	Me	E	13	729	724	1157	0.2
	20% throughfall reduction	*Arbutus unedo; Erica arborea; Fraxinus ornus*	Me	E	13	729	724	1415	
Talmon et al. (2011)	None	*Sarcopoterium spinosum*	Me	D	17.7	540		634	-0.06
	30% annual rainfall reduction	*Sarcopoterium spinosum*	Me	D	17.7	540		600	
	None	*Sarcopoterium spinosum*	Me	D	18.4	300		429	0.1
	30% annual rainfall reduction	*Sarcopoterium spinosum*	Me	D	18.4	300		472	
Carter et al. (2012)	None	*Calluna vulgaris; Vaccinium myrtillus; E. nigrum*	Te	D		1351		518	0.2
	Natural prolonged drought	*Calluna vulgaris; Vaccinium myrtillus; E. nigrum*	Te	D		1130		633	
	None	*Calluna vulgaris; Deschampsia flexuosa*	Te	D		668		732	-0.36
	Natural prolonged drought	*Calluna vulgaris; Deschampsia flexuosa*	Te	D		563		513	
	None	*Calluna vulgaris; Deschampsia flexuosa*	Te	D		714		651	-0.08

	Natural prolonged drought	*Calluna vulgaris; Deschampsia flexuosa*	Te	D				600	
	None	*Calluna vulgaris*	Te	D		986		310	-0.08
	Natural prolonged droughtt	*Calluna vulgaris*	Te	D		792		285	
	None	*Erica multiflora; Globularia alypum*	Te	D		550		390	-0.27
	Natural prolonged drought	*Erica multiflora; Globularia alypum*	Te	D			397	298	
Selsted et al. (2012)	None	*Calluna vulgaris; Deschampsia flexuosa*	Te	D	10		630	672	-0.1
	5-8% annual rainfall exclusion	*Calluna vulgaris; Deschampsia flexuosa*	Te	D	10		630	609	
	CO_2	*Calluna vulgaris; Deschampsia flexuosa*	Te	D	10		630	818	0.07
	5-8% annual rainfall exclusion, CO_2	*Calluna vulgaris; Deschampsia flexuosa*	Te	D	10		630	873	

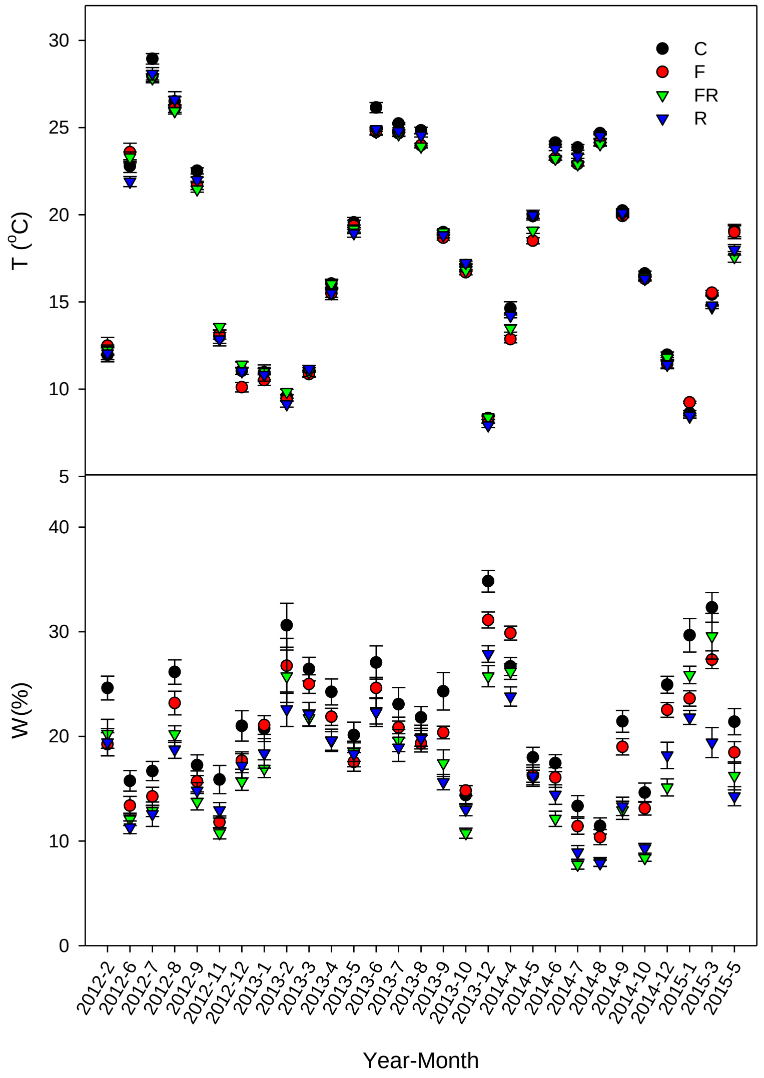

Figure 2.1. Seasonal patterns of soil temperature at 10cm soil depth (*T*) and volumetric water content at 20cm depth (*W*) during the three-year measurement period. The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

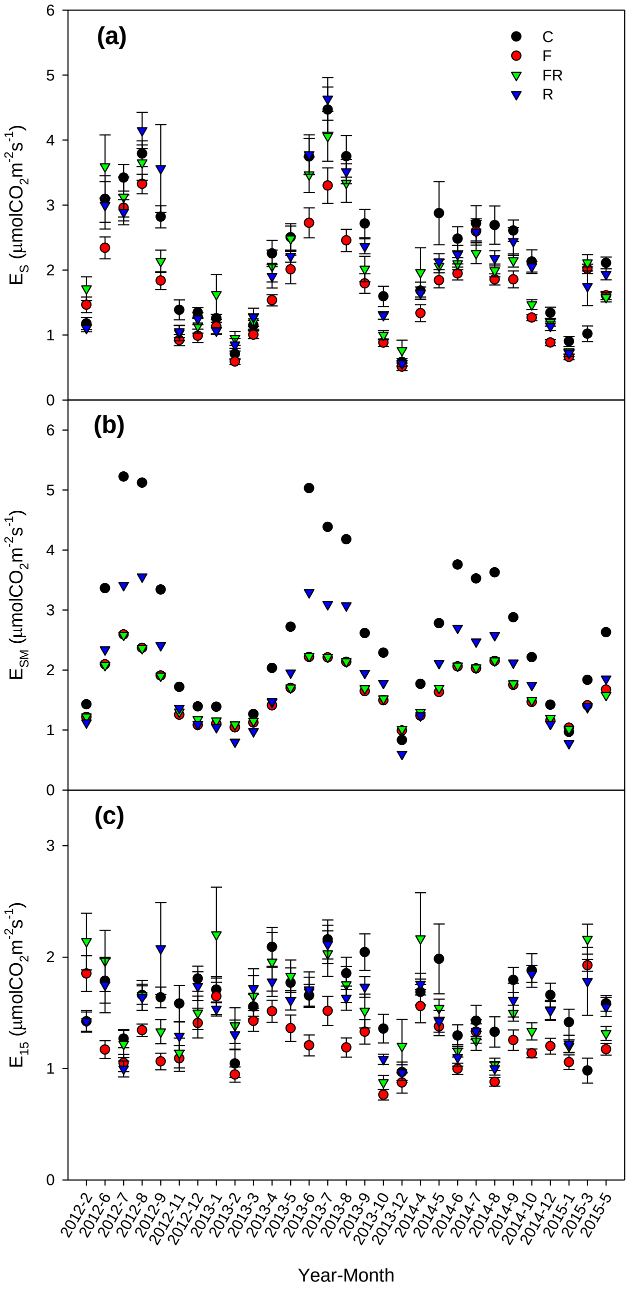

Figure 2.2. Seasonal patterns of soil CO_2 efflux (E_S), model soil CO_2 efflux (E_{SM}) and soil CO_2 efflux normalized to 15^{o}C (E_{15}). The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

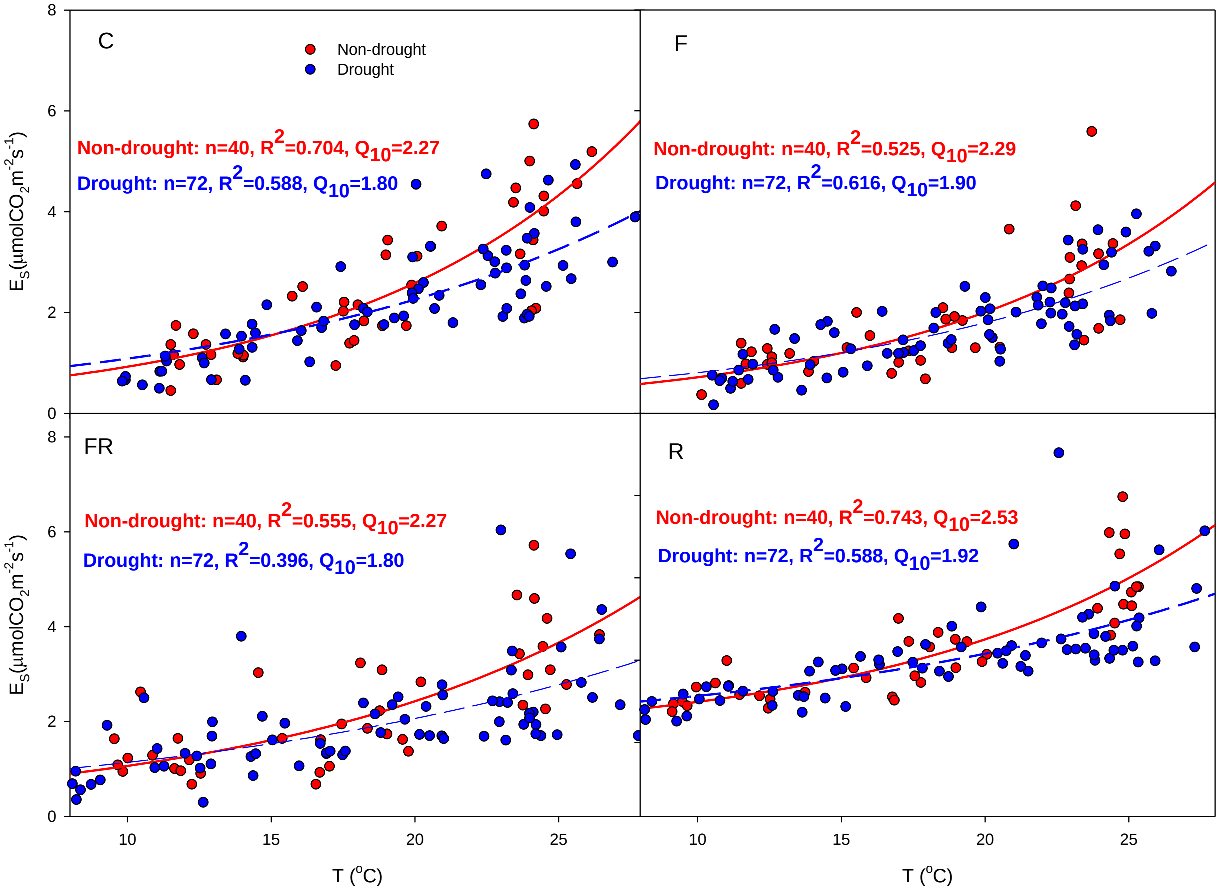

Figure 2.3. Relationships between soil CO_2 efflux (E_S) and soil temperature at 10cm soil depth (T) in drought and non-drought periods. The letter n indicates sample size, and R^2 indicates coefficient of determination. The significant level was α=0.0001. The red solid line is the regression between E_S and T in the non-drought period. The blue dashed line is the regression between E_S and T in the drought period. The abbreviations of four treatments are C- Control, F- fertilization only, FR- both fertilization and throughfall reduction, and R, throughfall reduction only.

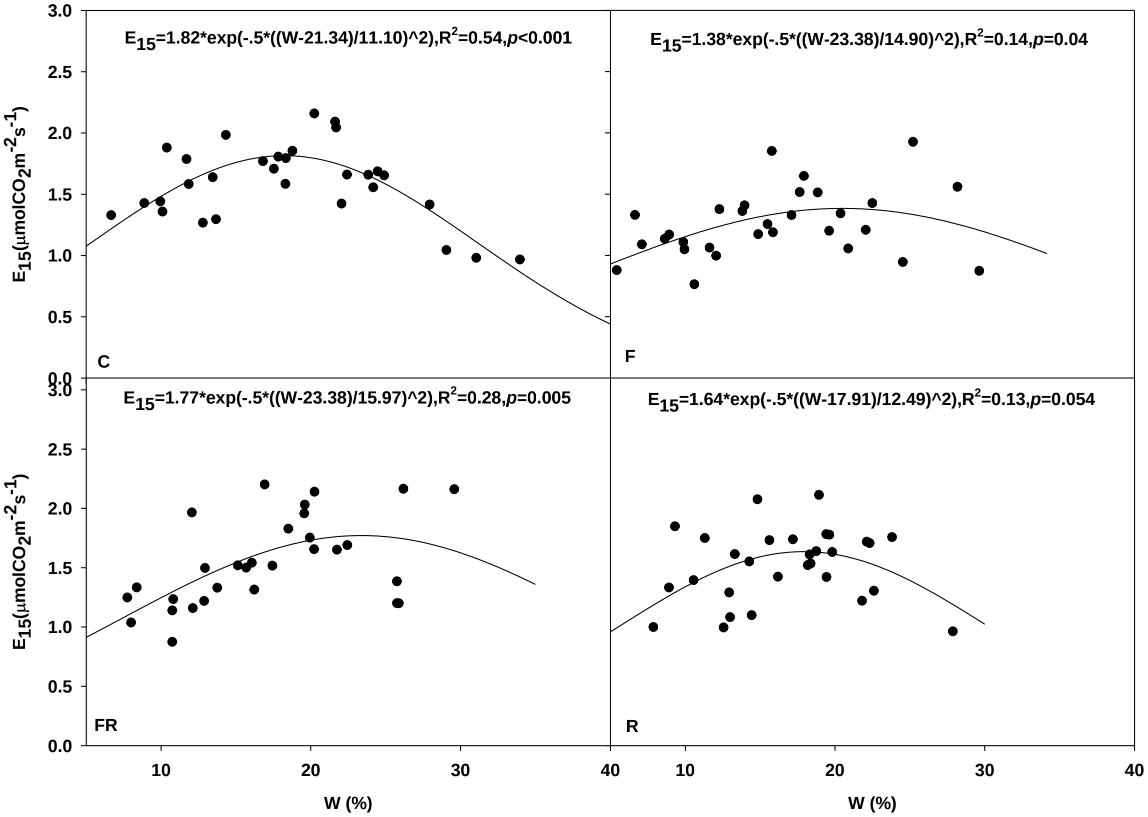

Figure 2.4. Relationships between soil CO_2 efflux normalized at 15°C (E_{15}) and volumetric water content at 20cm depth (*W*). The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

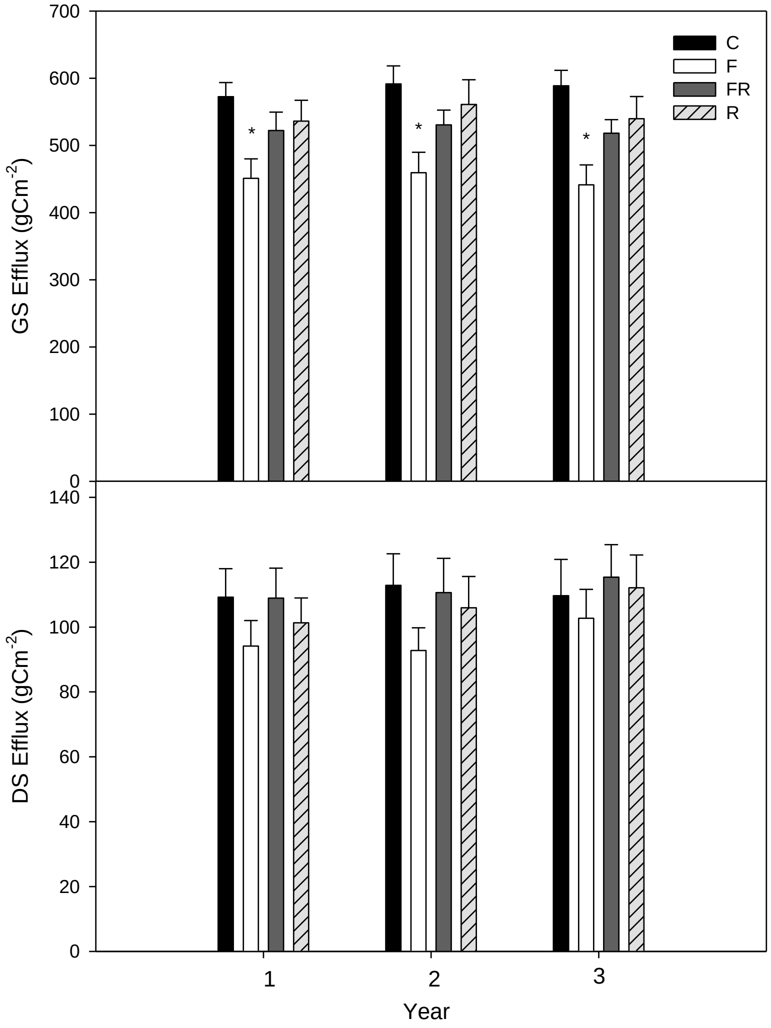

Figure 2.5. Cumulative soil CO_2 efflux (g C m^{-2}) in the growing seasons (GS) and the dormant seasons (DS) during the total 36 month measurement period. The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only. The asterisks indicate a significant difference between F and other treatments ($p<0.05$). Year represents periods of 12 months from June to May beginning after the installation of throughfall reduction and fertilization treatments in 2012.

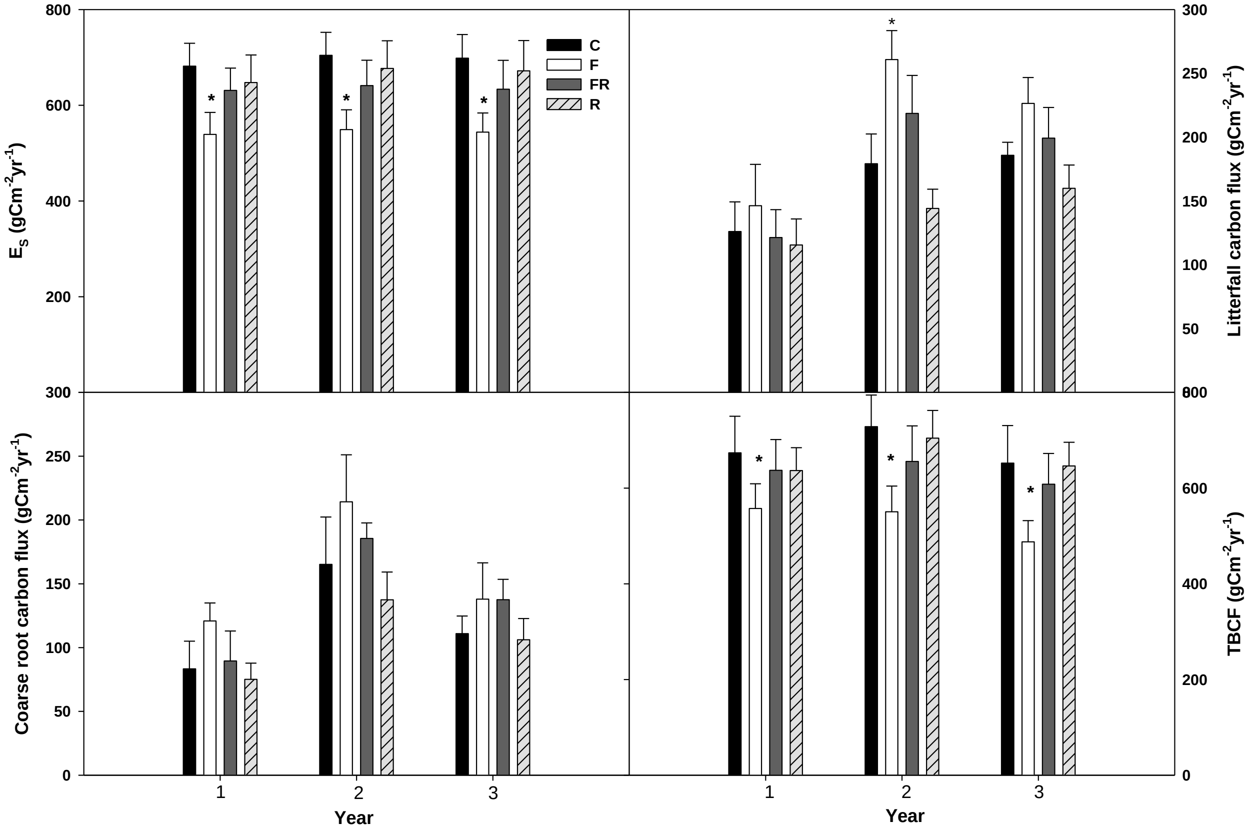

Figure 2.6. Soil CO_2 efflux (E_S) for the total 36 month measurement period, litterfall carbon flux, coarse root carbon flux and total belowground carbon flux (*TBCF*). The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only. The asterisks indicate there was a significant difference between F and other treatments ($p<0.05$). Year represents periods of 12 months from June to May beginning after the installation of throughfall reduction and fertilization treatments in 2012.

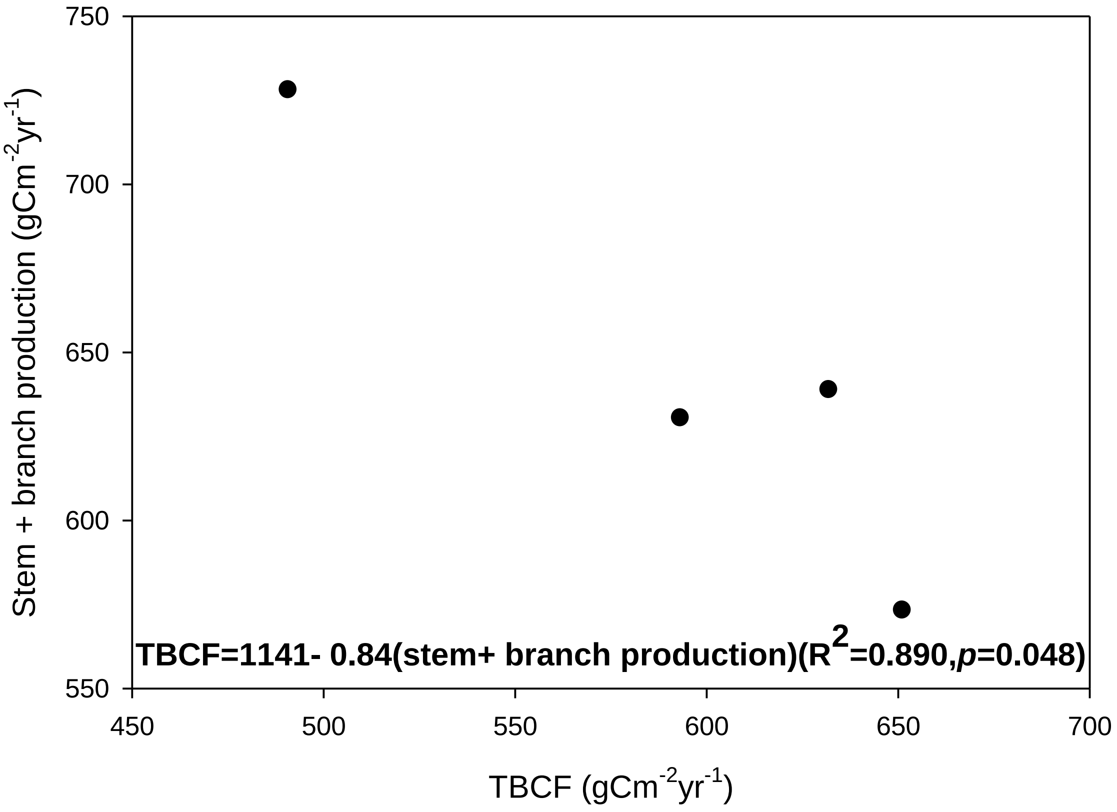

Figure 2.7. The relationship between stem and branch biomass production and total belowground carbon flux (*TBCF*).

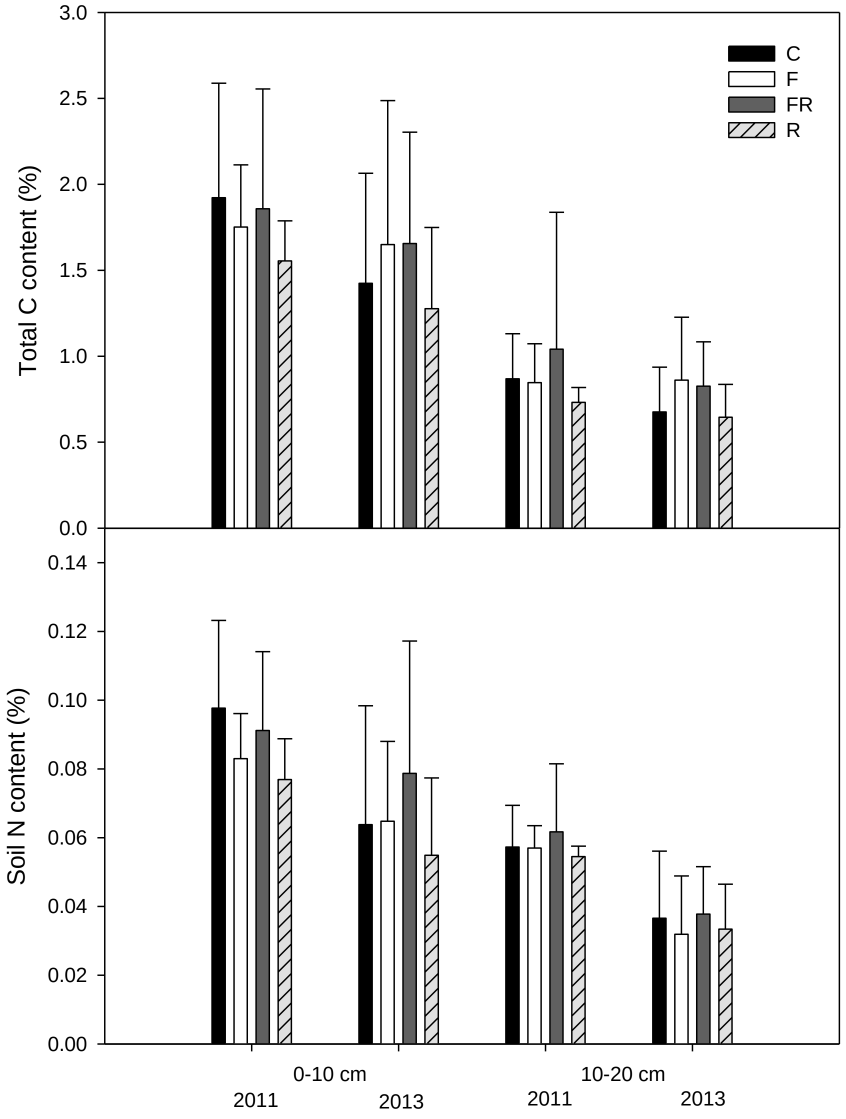

Figure S2.1. The total carbon (C) and nitrogen (N) content at two soil depth (between 0 cm and 10 cm and between 10 cm to 20 cm) in 2011 and 2013. The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

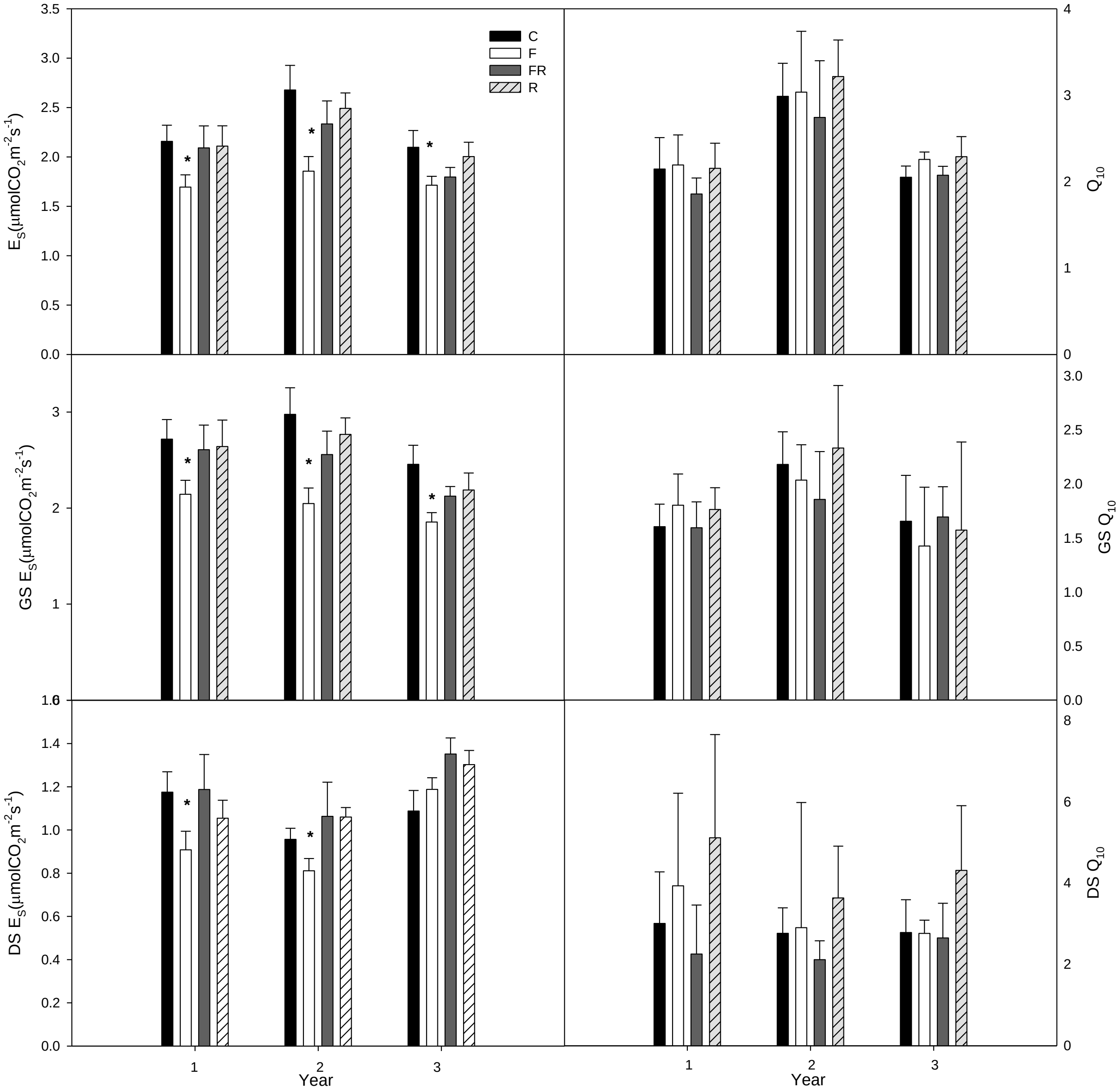

Figure S2.2. Means of soil CO_2 efflux (E_S) and its apparent temperature sensitivity (Q_{10}) for the total measurement period (upper plots) as well as in the growing season (GS) and the dormant season (DS). The asterisks indicate that there was a significant difference between F and other treatments ($p<0.05$). Year represents periods of 12 months from June to May beginning after the installation of throughfall reduction and fertilization treatments in 2012.

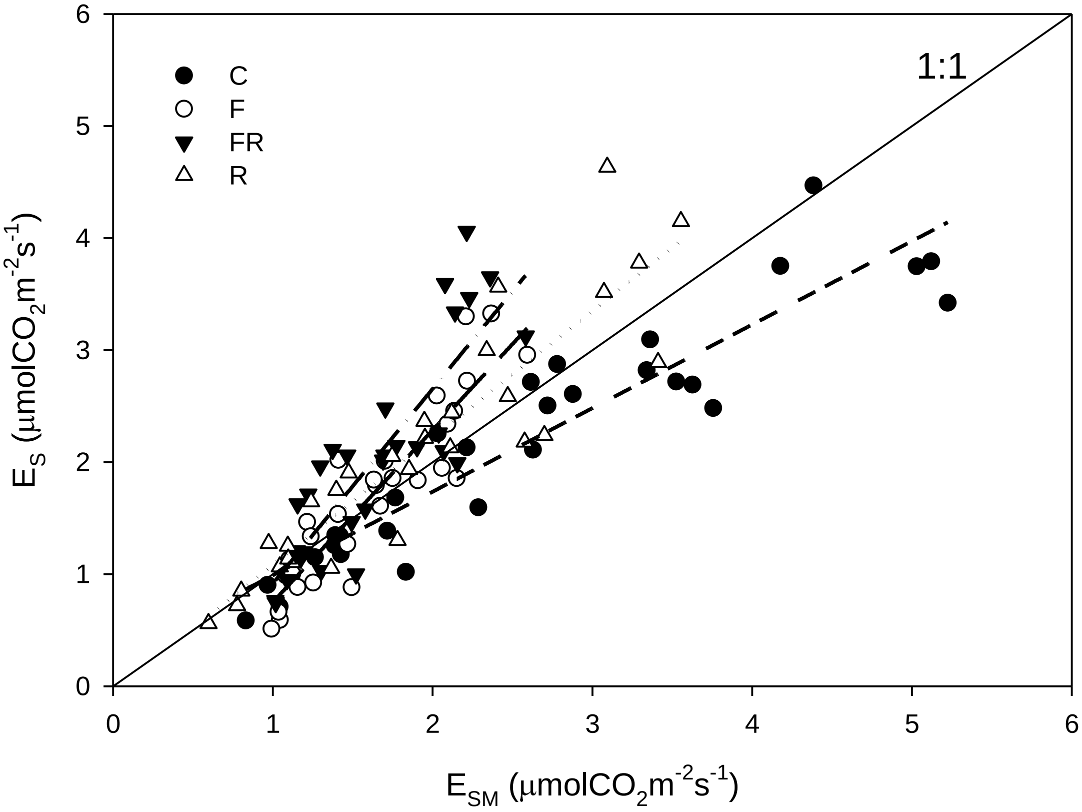

Figure S2.3. Linear regressions of between soil CO$_2$ efflux (*E*$_S$) and modeled soil CO$_2$ efflux (*E*$_{SM}$) during the three-year measurement period. The abbreviations of four treatments are C, control; F, fertilization only; FR, both fertilization and throughfall reduction; and R, throughfall reduction only. The solid line is the 1:1 line. The linear relationships for individual treatments are indicated by a short dashed line (C), a long dashed line (F), a dashed-dotted line (FR), and a dotted line (R).

References

Albaugh, T.J., Allen, H.L. & Fox, T.R. (2007) Historical patterns of forest fertilization in the southeastern United States from 1969 to 2004. *Southern Journal of Applied Forestry*, **31**, 129-137.

Albaugh, T.J., Allen, H.L. & Fox, T.R. (2008) Nutrient use and uptake in *Pinus taeda*. *Tree Physiology*, **28**, 1083-1098.

Albaugh, T.J., Allen, H.L., Dougherty, P.M., Kress, L.W. & King, J.S. (1998) Leaf area and above-and belowground growth responses of loblolly pine to nutrient and water additions. *Forest Science*, **44**, 317-328.

Bartkowiak, S.M., Samuelson, L.J., McGuire, M.A. & Teskey, R.O. (2015) Fertilization increases sensitivity of canopy stomatal conductance and transpiration to throughfall reduction in an 8-year-old loblolly pine plantation. *Forest Ecology and Management*, **354**, 87-96.

Binkley, D. (2002) Ten-year decomposition in a loblolly pine forest. *Canadian Journal of Forest Research*, **32**, 2231-2235.

Boden, T.A., Marland, G. & Andres, R.J. (2010) Global, regional, and national fossil-fuel CO_2 emissions. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tenn., USA doi,10.3334/CDIAC/00001_V2010

Bond-Lamberty, B. & Thomson, A. (2010a) Temperature-associated increases in the global soil respiration record. *Nature*, **464**, 579-582.

Bond-Lamberty, B. & Thomson, A. (2010b) A global database of soil respiration data. *Biogeosciences*, **7**, 1915-1926.

Borken, W., Savage, K., Davidson, E.A. & Trumbore, S.E. (2006) Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biology*, **12**, 177-193.

Brando, P.M., Nepstad, D.C., Davidson, E.A., Trumbore, S.E., Ray, D. & Camargo, P. (2008) Drought effects on litterfall, wood production and belowground carbon cycling in an Amazon forest: results of a throughfall reduction experiment. *Philosophical Transactionof the Royal Society London SeriesB Biological Sciences*, **363**, 1839-48.

Burton, A.J., Pregitzer, K.S., Crawford, J.N., Zogg, G.P. & Zak, D.R. (2004) Simulated chronic NO_3^- deposition reduces soil respiration in northern hardwood forests. *Global Change Biology*, **10**, 1080-1091.

Carter, M.S., Larsen, K.S., Emmett, B., Estiarte, M., Field, C., Leith, I.D., Lund, M., Meijide, A., Mills, R.T.E., Niinemets, Ü., Peñuelas, J., Portillo-Estrada, M., Schmidt, I.K., Selsted, M.B., Sheppard, L.J., Sowerby, A., Tietema, A. & Beier, C. (2012) Synthesizing greenhouse gas fluxes across nine European peatlands and shrublands – responses to climatic and environmental changes. *Biogeosciences*, **9**, 3739-3755.

Chen, G., Yang, Y., Guo, J., Xie, J. & Yang, Z. (2010) Relationships between carbon allocation and partitioning of soil respiration across world mature forests. *Plant Ecology*, **212**, 195-206.

Chen, G., Yang, Y. & Robinson, D. (2014) Allometric constraints on, and trade-offs in, belowground carbon allocation and their control of soil respiration across global forest ecosystems. *Global Change Biology*, **20**, 1674-1684.

Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, R., Jones, R., Kolli, R.K., Kwon, W. & Laprise, R. (2007) Regional climate projections. Climate Change, 2007:

The Physical Science Basis. Contribution of Working group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, University Press, Cambridge, Chapter 11, 847-940.

Cleveland, C.C., Wieder, W.R., Reed, S.C. & Townsend, A.R. (2010) Experimental drought in a tropical rain forest increases soil carbon dioxide losses to the atmosphere. *Ecology*, **91**, 2313-2323.

Coleman, M. (2007) Spatial and temporal patterns of root distribution in developing stands of four woody crop species grown with drip irrigation and fertilization. *Plant and Soil*, **299**, 195-213.

Cotrufo, M., Alberti, G., Inglima, I., Marjanović, H., LeCain, D., Zaldei, A., Peressotti, A. & Miglietta, F. (2011) Decreased summer drought affects plant productivity and soil carbon dynamics in Mediterranean woodland. *Biogeosciences*, **8**, 2729-2739.

Craine, J.M., Morrow, C. & Fierer, N. (2007) Microbial nitrogen limitation increases decomposition. *Ecology*, **88**, 2105-2113.

Daly, E., Palmroth, S., Stoy, P., Siqueira, M., Oishi, A.C., Juang, J., Oren, R., Porporato, A. & Katul, G.G. (2009) The effects of elevated atmospheric CO_2 and nitrogen amendments on subsurface CO_2 production and concentration dynamics in a maturing pine forest. *Biogeochemistry*, **94**, 271-287.

Davidson, E., Belk, E. & Boone, R.D. (1998) Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Global Change Biology*, **4**, 217-227.

Davidson, E.A. & Janssens, I.A. (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**, 165-173.

Davidson, E.A., Nepstad, D.C., Ishida, F.Y. & Brando, P.M. (2008) Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biology*, **14**, 2582-2590.

de Dato, G.D., De Angelis, P., Sirca, C. & Beier, C. (2010) Impact of drought and increasing temperatures on soil CO_2 emissions in a Mediterranean shrubland (*gariga*). *Plant and Soil*, **327**, 153-166.

Drake, J.E., Raetz, L.M., Davis, S.C. & DeLucia, E.H. (2010) Hydraulic limitation not declining nitrogen availability causes the age-related photosynthetic decline in loblolly pine (*Pinus taeda* L.). *Plant Cell &Environment*, **33**, 1756-66.

Drake, J.E., Oishi, A.C., Giasson, M.A., Oren, R., Johnsen, K.H. & Finzi, A.C. (2012) Trenching reduces soil heterotrophic activity in a loblolly pine (*Pinus taeda*) forest exposed to elevated atmospheric [CO_2] and N fertilization. *Agricultural and Forest Meteorology*, **165**, 43-52.

Egli, P., Maurer, S., Spinnler, D., Landolt, W., Günthardt-Goerg, M.S. & Körner, C. (2001) Downward adjustment of carbon fluxes at the biochemical, leaf, and ecosystem scale in beech-spruce model communities exposed to long-term atmospheric CO_2 enrichment. *Oikos*, **92**, 279-290.

Fischer, D.G., Hart, S.C., LeRoy, C.J. & Whitham, T.G. (2007) Variation in below-ground carbon fluxes along a *Populus* hybridization gradient. *New Phytologist*, **176**, 415-25.

Flanagan, L.B. & Johnson, B.G. (2005) Interacting effects of temperature, soil moisture and plant biomass production on ecosystem respiration in a northern temperate grassland. *Agricultural and Forest Meteorology*, **130**, 237-253.

Fontaine, S., Mariotti, A. & Abbadie, L. (2003) The priming effect of organic matter: a question of microbial competition? *Soil Biology and Biochemistry*, **35**, 837-843.

Ford, C.R., McGee, J., Scandellari, F., Hobbie, E.A. & Mitchell, R.J. (2012) Long- and short-term precipitation effects on soil CO_2 efflux and total belowground carbon allocation. *Agricultural and Forest Meteorology*, **156**, 54-64.

Fox, T.R., Allen, H.L., Albaugh, T.J., Rubilar, R. & Carlson, C.A. (2007) Tree nutrition and forest fertilization of pine plantations in the southern United States. *Southern Journal of Applied Forestry*, **31**, 5-11.

Gallardo, A. & Schlesinger, W.H. (1994) Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. *Soil Biology and Biochemistry*, **26**, 1409-1415.

Gallo, M., Amonette, R., Lauber, C., Sinsabaugh, R. & Zak, D. (2004) Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microbial Ecology*, **48**, 218-229.

George, K., Norby, R.J., Hamilton, J.G. & DeLucia, E.H. (2003) Fine-root respiration in a loblolly pine and sweetgum forest growing in elevated CO_2. *New Phytologist*, **160**, 511-522.

Giardina, C.P. & Ryan, M.G. (2002) Total Belowground Carbon Allocation in a Fast-growing Eucalyptus Plantation Estimated Using a Carbon Balance Approach. *Ecosystems*, **5**, 487-499.

Giardina, C.P., Litton, C.M., Crow, S.E. & Asner, G.P. (2014) Warming-related increases in soil CO_2 efflux are explained by increased below-ground carbon flux. *Nature Climate Change*, **4**, 822-827.

Giardina, C.P., Binkley, D., Ryan, M.G., Fownes, J.H. & Senock, R.S. (2004) Belowground carbon cycling in a humid tropical forest decreases with fertilization. *Oecologia*, **139**, 545-50.

Giardina, C.P., Coleman, M.D., Hancock, J.E., King, J.S., Lilleskov, E.A., Loya, W.M., Pregitzer, K.S., Ryan, M.G. & Trettin, C.C. (2005) The response of belowground carbon allocation in forests to global change. Tree species effects on soils: implications for global change, pp. 119-154. Springer.

Gonzalez-Benecke, C.A., Gezan, S.A., Albaugh, T.J., Allen, H.L., Burkhart, H.E., Fox, T.R., Jokela, E.J., Maier, C.A., Martin, T.A. & Rubilar, R.A. (2014) Local and general above-stump biomass functions for loblolly pine and slash pine trees. *Forest Ecology and Management*, **334**, 254-276.

Hanson, P.J., Edwards, N.T., Garten, C.T. & Andrews, J.A. (2000) Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry*, **48**, 115-146.

Hasselquist, N.J., Metcalfe, D.B. & Högberg, P. (2012) Contrasting effects of low and high nitrogen additions on soil CO_2 flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. *Global Change Biology*, **18**, 3596-3605.

Haynes, B.E. & Gower, S.T. (1995) Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiology*, **15**, 317-325.

Heinemeyer, A., Hartley, I.P., Evans, S.P., Carreira, D. L.A F., J.A. & Ineson, P. (2007) Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology*, **13**, 1786-1797.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M. & Read, D.J. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, **411**, 789-792.

Jackson, R.B., Cook, C.W., Pippen, J.S. & Palmer, S.M. (2009) Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology*, **90**, 3352-3366.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J. & Law, B.E. (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, **3**, 315-322.

Jassal, R.S., Black, T.A., Trofymow, J., Roy, R. & Nesic, Z. (2010) Soil CO_2 and N_2O flux dynamics in a nitrogen-fertilized Pacific Northwest Douglas-fir stand. *Geoderma*, **157**, 118-125.

Jia S.,Wand Z., Mei L., Sun Y.,Quan X.,Shi J.,Yu S.,Sun H., Gu J. (2007) Effect of nitrogen fertilization on soil respiration in Larix gmelinii and Fraxinus mandshurica plantations in China. Chinese *Journal of Plant Ecology*, **31**: 372-379.

Joslin, J., Wolfe, M. & Hanson, P. (2000) Effects of altered water regimes on forest root systems. *New Phytologist*, **147**, 117-129.

Karl, T.R. (2009) Global climate change impacts in the United States. Cambridge University Press.pp.14-27.

Kerhoulas, L.P., Kolb, T.E. & Koch, G.W. (2013) Tree size, stand density, and the source of water used across seasons by ponderosa pine in northern Arizona. *Forest Ecology and Management*, **289**, 425-433.

Kim, C. (2008) Soil carbon storage, litterfall and CO_2 efflux in fertilized and unfertilized larch (*Larix leptolepis*) plantations. *Ecological Research*, **23**, 757-763.

Lee, K.-H. & Jose, S. (2003) Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. *Forest Ecology and Management*, **185**, 263-273.

Li, H., Yan, J., Yue, X. & Wang, M. (2008) Significance of soil temperature and moisture for soil respiration in a Chinese mountain area. *Agricultural and Forest Meteorology*, **148**, 490-503.

Litton, C.M., Raich, J.W. & Ryan, M.G. (2007) Carbon allocation in forest ecosystems. *Global Change Biology*, **13**, 2089-2109.

Liu, L. & Greaver, T.L. (2010) A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters*, **13**, 819-28.

Liu, L., Wang, X., Lajeunesse, M.J., Miao, G., Piao, S., Wan, S., Wu, Y., Wang, Z., Yang, S., Li, P. & Deng, M. (2016) A cross-biome synthesis of soil respiration and its determinants under simulated precipitation changes. *Global Change Biology*, **22**, 1394-405.

Lu, M., Zhou, X., Luo, Y., Yang, Y., Fang, C., Chen, J. & Li, B. (2011) Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. *Agriculture, Ecosystems & Environment*, **140**, 234-244.

Maier, C.A. & Kress, L.W. (2000) Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability. *Canadian Journal of Forest Research*, **30**, 347-359.

Maier, C.A., Albaugh, T.J., Lee Allen, H. & Dougherty, P.M. (2004) Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: the effect of site resources on the stand carbon balance. *Global Change Biology*, **10**, 1335-1350.

Matamala, R. & Schlesinger, W.H. (2000) Effects of elevated atmospheric CO_2 on fine root production and activity in an intact temperate forest ecosystem. *Global Change Biology*, **6**, 967-979.

Matamala, R., Gonzalez-Meler, M.A., Jastrow, J.D., Norby, R.J. & Schlesinger, W.H. (2003) Impacts of fine root turnover on forest NPP and soil C sequestration potential. *Science*, **302**, 1385-1387.

McCarthy, H.R., Oren, R., Johnsen, K.H., Gallet-Budynek, A., Pritchard, S.G., Cook, C.W., LaDeau, S.L., Jackson, R.B. & Finzi, A.C. (2010) Re-assessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: interactions of atmospheric [CO_2] with nitrogen and water availability over stand development. *New Phytologist*, **185**, 514-528.

McCulley, R.L., Boutton, T.W. & Archer, S.R. (2007) Soil respiration in a subtropical savanna parkland: response to water additions. *Soil Science Society of America Journal*, **71**, 820-828.

McDowell, N.G., Balster, N.J. & Marshall, J.D. (2001) Belowground carbon allocation of Rocky Mountain Douglas-fir. *Canadian Journal of Forest Research*, **31**, 1425-1436.

Metcalfe, D.B., Meir, P., Aragao, L.E., Lobo-do-Vale, R., Galbraith, D., Fisher, R.A., Chaves, M.M., Maroco, J.P., da Costa, A.C., de Almeida, S.S., Braga, A.P., Goncalves, P.H., de Athaydes, J., da Costa, M., Portela, T.T., de Oliveira, A.A., Malhi, Y. & Williams, M. (2010) Shifts in plant respiration and carbon use efficiency at a large-scale drought experiment in the eastern Amazon. *New Phytologist*, **187**, 608-21.

Michel, K. & Matzner, E. (2003) Response of enzyme activities to nitrogen addition in forest floors of different C-to-N ratios. *Biology and Fertility of Soils*, **38**, 102-109.

Miller, A.T., Allen, H.L. & Maier, C.A. (2006) Quantifying the coarse-root biomass of intensively managed loblolly pine plantations. *Canadian Journal of Forest Research*, **36**, 12-22.

Misson, L., Limoudin, J., Rodriguez, R. & Letts, M.G. (2010) Leaf physiological responses to extreme droughts in Mediterranean *Quercus ilex* forest. *Plant, cell & environment*, **33**, 1898-1910.

Muhr, J. & Borken, W. (2009) Delayed recovery of soil respiration after wetting of dry soil further reduces C losses from a Norway spruce forest soil. *Journal of Geophysical Research: Biogeosciences*, **114** (G4).

Muhr, J., Borken, W. & Matzner, E. (2009) Effects of soil frost on soil respiration and its radiocarbon signature in a Norway spruce forest soil. *Global Change Biology,* **15**, 782-793.

Noormets, A., Gavazzi, M.J., McNulty, S.G., Domec, J.-C., Sun, G.E., King, J.S. & Chen, J. (2010) Response of carbon fluxes to drought in a coastal plain loblolly pine forest. *Global Change Biology*, **16**, 272-287.

Oishi, A.C., Palmroth, S., Johnsen, K.H., McCarthy, H.R. & Oren, R. (2014) Sustained effects of atmospheric [CO_2] and nitrogen availability on forest soil CO_2 efflux. *Global Change Biology,* **20**, 1146-60.

Palmroth, S., Oren, R., McCarthy, H.R., Johnsen, K.H., Finzi, A.C., Butnor, J.R., Ryan, M.G. & Schlesinger, W.H. (2006) Aboveground sink strength in forests controls the allocation of carbon below ground and its [CO2]-induced enhancement. *Proceedings of the National Academy of Sciences*, **103**, 19362-7.

Pangle, R.E. & Seiler, J. (2002) Influence of seedling roots, environmental factors and soil characteristics on soil CO2 efflux rates in a 2-year-old loblolly pine (Pinus taeda L.) plantation in the Virginia Piedmont. *Environmental Pollution*, **116**, S85-S96.

Phillips, R.P., Bernhardt, E.S. & Schlesinger, W.H. (2009) Elevated CO2 increases root exudation from loblolly pine (Pinus taeda) seedlings as an N-mediated response. *Tree physiology*, **29**, 1513-1523.

Pritchard, S.G., Strand, A.E., McCormack, M.L., Davis, M.A., Finzi, A.C., Jackson, R.B., Matamala, R., Rogers, H.H. & Oren, R.A.M. (2008) Fine root dynamics in a loblolly pine forest are influenced by free-air-CO2-enrichment: a six-year-minirhizotron study. *Global Change Biology*, **14**, 588-602.

Raich, J. & Nadelhoffer, K. (1989) Belowground carbon allocation in forest ecosystems: global trends. *Ecology*, **70**, 1346-1354.

Raich, J.W. & Schlesinger, W.H. (1992) The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus Series B-Chemical and Physical Meteorology*, **44**, 81-99.

Reich, P.B. & Bolstad, P. (2001) Productivity of evergreen and deciduous temperate forests. Terrestrial global productivity. Academic, San Diego, 245-283.

Rifai, S.W., Markewitz, D. & Borders, B. (2010) Twenty years of intensive fertilization and competing vegetation suppression in loblolly pine plantations: Impacts on soil C, N, and microbial biomass. *Soil Biology and Biochemistry*, **42**, 713-723.

Risk, D., Nickerson, N., Phillips, C.L., Kellman, L. & Moroni, M. (2012) Drought alters respired $\delta^{13}CO_2$ from autotrophic, but not heterotrophic soil respiration. *Soil Biology and Biochemistry*, **50**, 26-32.

Rodeghiero, M. & Cescatti, A. (2005) Main determinants of forest soil respiration along an elevation/temperature gradient in the Italian Alps. *Global Change Biology*, **11**, 1024-1041.

Rustad, L.E., Huntington, T.G. & Boone, R.D. (2000) Controls on soil respiration: implications for climate change. *Biogeochemistry*, **48**, 1-6.

Ryan, M.G. & Law, B.E. (2005) Interpreting, measuring, and modeling soil respiration. *Biogeochemistry*, **73**, 3-27.

Samuelson, L., Mathew, R., Stokes, T., Feng, Y., Aubrey, D. & Coleman, M. (2009) Soil and microbial respiration in a loblolly pine plantation in response to seven years of irrigation and fertilization. *Forest Ecology and Management*, **258**, 2431-2438.

Samuelson, L.J., Pell, C.J., Stokes, T.A., Bartkowiak, S.M., Akers, M.K., Kane, M., Markewitz, D., McGuire, M.A. & Teskey, R.O. (2014) Two-year throughfall and fertilization effects on leaf physiology and growth of loblolly pine in the Georgia Piedmont. *Forest Ecology and Management*, **330**, 29-37.

Schindlbacher, A., Wunderlich, S., Borken, W., Kitzler, B., Zechmeister-Boltenstern, S. & Jandl, R. (2012) Soil respiration under climate change: prolonged summer drought offsets soil warming effects. *Global Change Biology*, **18**, 2270-2279.

Scott-Denton, L.E., Rosenstiel, T.N. & Monson, R.K. (2006) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biology*, **12**, 205-216.

Seager, R., Tzanova, A. & Nakamura, J. (2009) Drought in the Southeastern United States: Causes, Variability over the Last Millennium, and the Potential for Future Hydroclimate Change. *Journal of Climate*, **22**, 5021-5045.

Selsted, M.B., Linden, L., Ibrom, A., Michelsen, A., Larsen, K.S., Pedersen, J.K., Mikkelsen, T.N., Pilegaard, K., Beier, C. & Ambus, P. (2012) Soil respiration is stimulated by elevated CO_2 and reduced by summer drought: three years of measurements in a multifactor ecosystem manipulation experiment in a temperate heathland (CLIMAITE). *Global Change Biology*, **18**, 1216-1230.

Shi, Z., Thomey, M.L., Mowll, W., Litvak, M., Brunsell, N.A., Collins, S.L., Pockman, W.T., Smith, M.D., Knapp, A.K. & Luo, Y. (2014) Differential effects of extreme drought on production and respiration: synthesis and modeling analysis. *Biogeosciences*, **11**, 621-633.

Sotta, E.D., Veldkamp, E., Schwendenmann, L., Guimaraes, B.R., Paixao, R.K., Ruivo, da COSTA M., Calos L., Arlos, A. & Meir, P. (2007) Effects of an induced drought on soil carbon dioxide (CO_2) efflux and soil CO_2 production in an Eastern Amazonian rainforest, Brazil. *Global Change Biology*, **13**, 2218-2229.

Sowerby, A., Emmett, B.A., Tietema, A. & Beier, C. (2008) Contrasting effects of repeated summer drought on soil carbon efflux in hydric and mesic heathland soils. *Global Change Biology*, **14**, 2388-2404.

Straaten, O.v., Veldkamp, E., Köhler, M. & Anas, I. (2010) Spatial and temporal effects of drought on soil CO_2 efflux in a cacao agroforestry system in Sulawesi, Indonesia. *Biogeosciences*, **7**, 1223-1235.

Subke, J.A., Inglima, I. & Cotrufo, M.F. (2006) Trends and methodological impacts in soil CO_2 efflux partitioning: A metaanalytical review. *Global Change Biology*, **12**, 921-943.

Suseela, V. & Dukes, J.S. (2013) The responses of soil and rhizosphere respiration to simulated climatic changes vary by season. *Ecology*, **94**, 403-413.

Suseela, V., Conant, R.T., Wallenstein, M.D. & Dukes, J.S. (2012) Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Global Change Biology*, **18**, 336-348.

Talmon, Y., Sternberg, M. & Gruenzweig, J.M. (2011) Impact of rainfall manipulations and biotic controls on soil respiration in Mediterranean and desert ecosystems along an aridity gradient. *Global Change Biology*, **17**, 1108-1118.

Tang, J.W. & Baldocchi, D.D. (2005) Spatial-temporal variation in soil respiration in an oak-grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components. *Biogeochemistry*, **73**, 183-207.

Teste, F.P., Lieffers, V.J. & Strelkov, S.E. (2012) Ectomycorrhizal community responses to intensive forest management: thinning alters impacts of fertilization. *Plant and Soil*, **360**, 333-347.

Treseder, K.K. (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters*, **11**, 1111-20.

Tyree, M.C., Seiler, J.R., Aust, W.M., Sampson, D.A. & Fox, T.R. (2006) Long-term effects of site preparation and fertilization on total soil CO_2 efflux and heterotrophic respiration in a 33-year-old *Pinus taeda* L. plantation on the wet flats of the Virginia Lower Coastal Plain. *Forest Ecology and Management*, **234**, 363-369.

Vallack, H.W., Leronni, V., Metcalfe, D.B., Högberg, P., Ineson, P. & Subke, J.-A. (2012) Application of nitrogen fertilizer to a boreal pine forest has a negative impact on the respiration of ectomycorrhizal hyphae. *Plant and Soil*, **352**, 405-417.

Vogel, J.G., Bond-Lamberty, B.P., Schuur, E.A.G., Gower, S.T., Mack, M.C., O'Connell, K.E.B., Valentine, D.W. & Ruess, R.W. (2008) Carbon allocation in boreal black spruce forests across regions varying in soil temperature and precipitation. *Global Change Biology*, **14**, 1503-1516.

Wang, C., Yang, J. & Zhang, Q. (2006) Soil respiration in six temperate forests in China. *Global Change Biology*, **12**, 2103-2114.

Wei, W., Weile, C. & Shaopeng, W. (2010) Forest soil respiration and its heterotrophic and autotrophic components: Global patterns and responses to temperature and precipitation. *Soil Biology and Biochemistry*, **42**, 1236-1244.

Will, R.E., Markewitz, D., Hendrick, R.L., Meason, D.F., Crocker, T.R. & Borders, B.E. (2006) Nitrogen and phosphorus dynamics for 13-year-old loblolly pine stands receiving complete competition control and annual N fertilizer. *Forest Ecology and Management*, **227**, 155-168.

Will, R.E., Fox, T., Akers, M., Domec, J.-C., González-Benecke, C., Jokela, E.J., Kane, M., Laviner, M.A., Lokuta, G. & Markewitz, D. (2015) A range-wide experiment to investigate nutrient and soil moisture interactions in loblolly pine plantations. *Forests*, **6**, 2014-2028.

Williams, J.W., Jackson, S.T. & Kutzbach, J.E. (2007) Projected distributions of novel and disappearing climates by 2100 AD. *Proceedings of the National Academy of Sciences*, **104**, 5738-5742.

Wu, Z., Dijkstra, P., Koch, G.W., PeÑUelas, J. & Hungate, B.A. (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology*, **17**, 927-942.

Yan, L., Chen, S., Huang, J. & Lin, G. (2010) Differential responses of auto- and heterotrophic soil respiration to water and nitrogen addition in a semiarid temperate steppe. *Global Change Biology*, **16**, 2345-2357.

Zak, D.R., Pregitzer, K.S., Burton, A.J., Edwards, I.P. & Kellner, H. (2011) Microbial responses to a changing environment: implications for the future functioning of terrestrial ecosystems. *Fungal Ecology*, **4**, 386-395.

Zhang, Y., Majumdar, I. & Schelhas, J. (2010) Changes in woodland use from longleaf pine to loblolly pine. *Sustainability*, **2**, 2734-2745.

Zhao, D., Kane, M., Teskey, R., Markewitz, D., Greene, D. & Borders, B. (2014) Impact of management on nutrients, carbon, and energy in aboveground biomass components of mid-rotation loblolly pine (*Pinus taeda* L.) plantations. *Annals of Forest Science*, **71**, 843-851.

Zhou, L., Zhou, X., Zhang, B., Lu, M., Luo, Y., Liu, L. & Li, B. (2014) Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. *Global Change Biology*, **20**, 2332-43.

CHAPTER 3

EFFECTS OF FERTILIZATION AND THROUGHFALL REDUCTION ON CO2 EFFLUX FROM ROOTS, HETEROTROPHIC AND ECTOMYCORRHIZAL HYPHAE IN A LOBLOLLY PINE (*PINUS TAEDA*) PLANTATION[2]

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Abstract

Soil CO_2 efflux (E_S) is an important component of forest ecosystem carbon budgets and net ecosystem CO_2 exchange. The response of E_S and its components to changes in soil fertility and decreasing soil moisture are crucial to estimating carbon balance and exchange in forests under management in future climatic regimes. The experiment design was a 2 × 2 factorial combination of fertilization (control and fertilized) and precipitation (control and a 30% throughfall reduction) replicated in four blocks. We measured E_S along with soil temperature (T) and soil moisture (W) from 2014 to 2015 in a loblolly pine plantation in Washington, GA. Heterotrophic CO_2 efflux (E_H), root CO_2 efflux (E_R) and CO_2 efflux from ectomycorrhizal hyphae (E_M) were separated using trenched and untrenched plots. Our objectives were to (1) examine how soil temperature, moisture and *LAI* affect E_S, E_H, E_R and E_M; (2) quantify annual, growing and dormant season E_S, E_H, E_R and E_M; and (3) determine the effects of fertilization and throughfall reduction on E_S, E_H, E_R and E_M. Our hypotheses were: (1) fertilization decreases E_H; (2) throughfall reduction decreases E_R; and (3) *LAI* was positively related with E_H and E_R. We found that fertilization reduced E_S and E_R while a 30% reduction in throughfall had no effect on E_H, E_R and E_M. The decrease in E_R may be related to a shift in the production from fine roots to coarse roots. Soil temperature and moisture, and their interaction, explained less variation in E_R and E_M than in E_S and E_H in the four treatments. Soil CO_2 efflux normalized to 15^oC (E_{S15}) increased with increasing soil moisture in control (C), fertilization (F) and the interaction between fertilization and thourghfall reduction treatments. Heterotrophic CO_2 efflux normalized at $15\ ^oC$ (E_{H15}) also increased with increasing soil moisture in C and F treatments. Soil temperature explained more variation in E_S, E_H and E_R in non-drought periods compared to drought periods. The correlation between E_H and *LAI* indicates a potential relationship between

the gross primary productivity and E_S. The contribution of E_H to E_S ranged from 50% to 58% among the treatments. The contribution of E_M to E_S ranged from 10% to 12% among the treatments. The quantification of E_M in temperate forests will improve our understanding of how E_R will respond to management practices and future changes in climate. The effect of decreased precipitation on E_S and its component fluxes should be cautiously interpreted due to complicated interactions with soil temperature, leaf area index and soil water availability.

Introduction

The southeastern United States has roughly 21.8 million hectares of loblolly and shortleaf pine forest plantations which are regarded as some of the most intensively managed forests in the world (Fox et al., 2007). Fertilization is the most common practice used to increase loblolly pine productivity (Fox et al., 2007). It has also been predicted that summertime precipitation will decline by 10 to 30% in the southeastern region (Christensen et al., 2007). Both fertilization and decreases in soil moisture have the potential to change soil CO_2 efflux (E_S), which is a major portion of the CO_2 entering the atmosphere in these forest ecosystems.

Forest soil CO_2 efflux can be separated into autotrophic (E_A) and heterotrophic (E_H) components (Bond-Lamberty et al., 2004; Subke et al., 2006; Zhou et al., 2014; Liu et al., 2016). The autotrophic component is composed of CO_2 efflux from roots and mycorrhizal fungi. Although it is difficult to separately measure those effluxes, it is possible to separate the CO_2 efflux of roots (E_R) from that of mycorrhizal hyphae (E_M). The contribution of ectomycorrhizal hyphae to E_S in some boreal and temperate forests has been reported to be between 8 and 33% (Hasselquist et al., 2012; Fahey et al., 2005; Langley et al., 2006; Heinemeyer et al., 2007; Heinemeyer et al., 2011; Vallack et al., 2012; Andrew et al., 2014; Neumann and Matzner, 2014). The heterotrophic component of soil CO_2 efflux consists of the metabolism of free living microorganisms, and has been estimated to contribute from 40% to 65% to E_S in different forest ecosystems (Hanson et al., 2000; Subke et al., 2006).

Several meta-analyses have concluded that fertilization decreases E_H in temperate forests (Lichter et al., 2008; Janssens et al., 2010; Zhou et al., 2014). Other studies reported that nitrogen fertilization led to reductions in both E_A and E_H due to less fine root production and less mycorrhizal colonization of roots (Janssens et al., 2010; Olsson et al., 2005). Fertilization decreased E_A in older loblolly pine stands that were subjected to repeated fertilizations over a seven year period (Samuelson et al., 2009). Samuelson et al. (2009) concluded that a reduction in fine root biomass was responsible for the decrease in E_A. However, fertilization increased E_A in young loblolly pine plantations (less than 3-years old) (Tyree et al., 2008; Gough and Seiler, 2004). It has also been reported that fertilization had no effect on E_A in loblolly pine plantations. Drake et al. (2008) found that fertilization had no significant effect on *in situ* fine root respiration scaled to the stand level in a loblolly pine plantation. Maier and Kress (2000) also found no effects of fertilization on E_A in an 11-year-old loblolly pine plantation. A negative effect of fertilization on E_M, caused by a reduction in fungal colonization or through fungal malfunction, has been reported in several studies (Teste et al., 2012; Hasselqusit et al., 2012; Vallack et al., 2012).

The Southeast region of the US is prone to moderate decreases in water availability (precipitation minus evapotranspiration), and there is likely to be more variation in precipitation in the future (Kunkel et al., 2015; Seager et al., 2009). Soil water deficit induced by decreased precipitation can impact E_S and its components. Many field studies have shown that dry soil conditions suppressed E_S (Suseela et al., 2012; Risk et al., 2012; Schindlbacher et al., 2012; Heinemeyer et al., 2012). However, the component of E_S (E_R, E_H or E_M) responsible for the decrease in E_S remains unclear. Drought has been shown to reduce E_H more than E_R (Scott-Denton, 2006; Borken et al., 2006; Noormets et al., 2010; Suseela et al., 2012), less than E_R (Cudlín et al. 2007; Risk et al., 2012), or reduce both E_R and E_H similarly (Schindlbacher et al. 2012).   One reason for these different findings is that some microbial species can physiologically adapt to lower water potentials (Schimel et al., 2007; Williams, 2007). In particular, soil fungi may be better adapted to water stress than bacteria or microfauna in soil and help keep E_H stable as soils dry (Yuste et al., 2011; De Vries et al., 2012). Consistent with that, Risk et al. (2012) demonstrated that temporal variation of E_S was associated with E_A in five forest sites in eastern Canada. However, Schindlbacher et al. (2012) showed that drought reduced E_A and E_H to the same degree and microbial biomass was not affected by the treatment. Recently, Liu et al. (2016) reported that that there was no reduction in E_S, E_H or E_A under decreased precipitation treatments in studies on 13 temperate forests, indicating that the level of soil and plant water deficit is critical to how determining much the fluxes are affected by drought.

Close correlations between E_S and gross primary productivity (*GPP*) have been reported in forest ecosystems globally (Litton et al., 2007; Bond-lamberty et al., 2004; Hopkins et al., 2012; Chen et al., 2014). Leaf area index (*LAI*), a proxy of *GPP*, has been reported to be correlated with E_S in forest ecosystems (Lindroth et al., 2008; Reichstein 2003; Curiel Yuste et al., 2004; Hibbard et al., 2005; Oishi et al., 2013). Positive (Reichstein 2003; Hibbard et al., 2005; Oishi et al., 2013) or negative (Curiel Yustie et al., 2004; Lindroth et al., 2008; Ngao et al., 2012) correlations between E_S and *LAI* have been reported in both deciduous and coniferous temperate forests. Little is known about the correlation between E_A, or E_H and *LAI*. Only one study has reported a relationship between E_H and *LAI*. Templeton (2009) reported that there was a negative relationship between an index of E_H (square root transformed E_H) and *LAI* in loblolly pine plantations. No studies have reported correlations between E_A and *LAI* in forests.

This study was conducted in a loblolly pine plantation in East-central Georgia. Our objectives were to (1) examine how soil temperature, moisture and *LAI* affect E_S, E_H, E_R and E_M; (2) quantify annual, growing and dormant season E_S, E_H, E_R and E_M; and (3) determine the effects of fertilization and throughfall reduction on E_S, E_H, E_R and E_M. Our hypotheses are: (1)

fertilization decreases E_H; (2) throughfall reduction decreases E_R; and (3) *LAI* is positively related with E_H and E_R.

Materials and Methods

Site description and experimental design

The study site was a loblolly pine plantation in Taliaferro County, near Washington, GA (33°37´32.61´´ N, 82°47´ 56.54´´ W). The average mean annual temperature is 16.1°C and mean annual precipitation is 1109 mm. The precipitation was 304mm higher than the average annual precipitation in 2013, 120 mm lower than the mean annual precipitation in 2014 and 167 mm higher in 2015. The soil surface and subsoil texture is clay loam and clay, respectively. The soil pH was 4.84 before treatment. The stand was 8-years-old in 2013 and stand density was 1373 trees ha^{-1}, mean tree diameter at breast height (DBH) was 13.4 cm and mean stand LAI was 3.0 in 2014 (Table 3.1).

The 2 × 2 factorial combination of fertilization and throughfall reduction was replicated in four blocks. There were 4 replicate plots for each treatment and 16 plots in total. The treatment plots were 1000 m^2 (34.1m × 28.0 m) and the central measurement plots were 300 m^2 (21.3 m × 14.0 m). The treatments were control (C): no treatments applied; fertilization (F): a one-time fertilizer application at the beginning of the study (2012); throughfall reduction (R): a 30% reduction in throughfall; and fertilization plus throughfall reduction (FR): the one-time fertilizer application combined with the 30% reduction in throughfall. The fertilizer application consisted of 224 kg N ha^{-1}, 28 kg P ha^{-1} (N and P were applied as a mixture of urea: 432 kg ha $^{-1}$ and diammonium phosphate: 140 kg ha $^{-1}$), 56 kg K ha^{-1} (potassium chloride) and a micronutrient blend (22.4 kg ha^{-1}). This fertilization was based on prior measurements of optimum foliar nutrient concentrations in loblolly pine plantations (Albaugh et al., 2008). The throughfall reduction troughs covered 30% of the ground area. They were installed between tree rows in each plot of the R and FR treatments in May, 2012. A wood frame was constructed (1.8 m wide and 1.3 m high) to support two layers of U.V. stabilized coextruded polyethylene with embedded high strength cord (Polyscrim 12, Americover Inc., Escondido, CA,USA). This structure collected throughfall and drained it from the plots. More detailed information about the throughfall reduction troughs can be found in Samuelson et al. (2014). The leaf and branch litter that accumulated in the troughs was manually removed frequently and distributed within the plot to avoid an uneven distribution of detritus on the forest floor.

Measurement of soil CO_2 efflux

We randomly inserted four PVC collars (diameter 10 cm, height 5 cm) directly through the litter layer in each plot one week before the first measurements which became the E_S measurement locations for the study. We measured instantaneous E_S using a soil chamber (LI-6400-09) connected to a portable infrared gas analyzer (LI-6400, Li-Cor, Inc., Lincoln, NE, USA). Simultaneously, soil temperature at 10cm depth (T) was measured with a soil thermocouple probe (6000-09TC Soil Temperature Probe, LICOR Inc., Lincoln, NE, USA) within 5 cm of the measurement collar. Soil moisture at 20 cm depth (W) was measured near the soil collar using a HydroSense™ soil water content sensor (Campbell Scientific Inc, Logan, UT and Campbell Scientific Australia, Townsville, QLD, Australia). We took monthly measurements from April, 2014 to December, 2015. Continuous soil temperature at 10cm depth (107-L Thermistor, Campbell Scientific, Logan, UT, USA) and moisture at 20cm depth (Wireless Soil Water Reflectometer, CWS655, Campbell Scientific, Logan, Utah, USA) were measured by a weather station at the site as described in Bartkowiak et al. (2015).

Partitioning E_{H}, E_{R} and E_{M}

We used a combination of thick plastic sheeting and mesh screen lining around trenched subplots to separate E_{H}, E_{R} and E_{M}. Two trenched subplots (30 × 30 cm and 40 cm deep) were edged with thick plastic which prevented the growth of roots and mycorrhizal hyphae and were used to estimate E_{H} in each plot. An additional four trenched subplots (30cm × 30 cm × 40 cm) were edged with mesh screen with a 42 µm pore size. The mesh screen allowed in-growth by mycorrhizal hyphae but not fine roots. The CO_2 efflux from these subplots included E_{M} and E_{H} ($E_{\mathrm{H+M}}$). We used the difference in CO_2 efflux between untrenched plots and trenched subplots edged with mesh screen to estimate $E_{\mathrm{R}} = E_{\mathrm{S}} - E_{\mathrm{H+M}}$. We used the difference between the subplots edged with thick plastic and those lined with mesh screen to estimate E_{M}. In total, there were 32 subplots for E_{H} and 64 subplots for E_{M}. All subplots were trenched before November, 2013. To minimize any potential effects from disturbance of the soil and decomposition of dead roots after trenching, we did not began the monthly efflux measurements until April, 2014, six months after the trenching took place. Any plant growth in trenched subplots was removed before measurements took place. The effect of trenching on soil moisture was tested and there were no significant differences in soil moisture in any of the treatments (Figure S3.1). The differences in soil moisture between untrenched plots and the two types of trenched subplots ($E_{\mathrm{H+M}}$ and E_{H}) ranged from - 4% to 5%.

In a separate experiment in the C plots, we examined whether the volume of the trenched subplot affected the estimate of E_{H}. Three different root exclusion subplots were used: 1) 10 cm diameter × 40 cm deep electric conduit tube driven into the soil, 2) 30 cm × 30 cm × 40 cm deep trenched subplots lined with plastic and 3) 50 cm × 50 cm × 40 cm deep trenched subplots lined with plastic. These treatments were established in November, 2013. There were three replicates for each trenching area treatment. There was no significant difference in CO_2 efflux from the subplots between the trenched subplots that were 30 cm × 30 cm x 40cm or 50cm × 50cm x 40cm. However, there was more fluctuation in the CO_2 efflux from the electric conduit (10 cm x 40 cm) than in the other two trenching treatments (Fig. S3.2). In addition, it was not possible to take measurements in the conduits in August and December, 2015 due to water accumulation in the conduits.

To determine the link between the stem CO_2 efflux (E_{STEM}) and root CO_2 efflux, we measured E_{STEM} on four individual trees in each plot. The distance between these trees and the trenched subplots ($E_{\text{H+M}}$ and E_{H}) was less than 1m. The LI-6400 portable gas exchange system with a LI-6400-09 chamber (Li-Cor Inc, Lincoln, NE, USA) was used to measure E_{STEM}. This approach has been used by others using the LI-6400 system (Xu et al., 2000; Moore et al., 2008). For each measurement the chamber was placed on an opaque PVC collar (10 cm inside diameter), permanently mounted to the tree stem at the 1.2 m above the ground. The loose and dead bark was carefully removed from each sampled tree with a brush without damaging the underlying cambium before installing the PVC collars in March, 2014. We used generic 100% silicon adhesive (Clear Silicon 1*, Momentive Performance Materials Inc., Huntersville, NC, USA) to adhere the collars to the tree. The area and volume of each collar were determined and leak tests were performed to ensure the chambers were air-tight. The collars were directly open to air except during each measurement (3 to 4 minutes each time). We measured E_{STEM} monthly, the same day that the E_{S}, E_{H} and $E_{\text{H+M}}$ measurements were made, from April, 2014 to December, 2015. Concurrent with E_{STEM} measurements, we also measured stem temperature at 1 cm depth with a Li-6000-09-TC soil probe thermocouple (Li-Cor Inc, Lincoln, NE, USA).

Aboveground net primary production and leaf area index measurement

We measured DBH and height of all trees (living and dead) in each replicated plot in December from 2011 to 2014. We used allometric equations developed for loblolly pine to estimate the stem, branch, foliage and bark biomass (Gonzalez-Benecke et al. 2014). Woody biomass production (stem + branch) was assumed to be equal to the net increment in woody biomass in the measurement interval.

We installed 12 round litter traps (area=0.5 m^2) in each plot. The litter was collected each month from January, 2014 to November, 2015. The litter in the traps was collected and oven-dried at 70°C to constant mass every month. Simultaneously, we took monthly leaf area index (*LAI*) measurements with a LAI-2000 Plant Canopy Analyzer (Li-Cor, Inc., Lincoln, NE, USA) in each plot from January, 2014 to November, 2015.

Data analysis

We assessed potential relationships between T and W with CO_2 efflux (E_S, E_H, E_R and E_M) using the multiple regression procedure of SAS (Version 8.2; SAS Inc., Cary, NC, USA). We performed logarithmic transformations of E_S, E_H, E_R and E_M to achieve linearity and homoscedasticity. We used backward elimination to remove insignificant terms ($\alpha > 0.05$). The final form of the regression model was: $\mathrm{Ln}\,(E) = a + b\,T + c\,W + d\,T \times W$. The model $\mathrm{Ln}\,(E) = a + bT$ was used to calculate Q_{10} using the equation $Q_{10} = e^{10b}$. The overall means of E_S, E_H, E_R, E_M and Q_{10} of each treatment were compared with ANCOVA and Tukey’s multiple range tests. On a daily time step, we used the regression models (Table 3.4) and continuous soil temperature and moisture provided by the weather station to estimate the cumulative E_S, E_H, E_R and E_M in each month in the growing season and dormant season as well as the annual cumulative fluxes. We also determined the monthly cumulative E_R contribution (E_{RC} /E_{SC}) to monthly E_S. We also normalized the E_S, E_H, E_R, E_M and E_{STEM} at 15°C.

A two –way randomized complete block design analysis of variance (ANOVA) was performed for all the measurements using PROC GLM multiple regression procedure of SAS (Version 8.2; SAS Inc., Cary, NC, USA). Each plot was an experiment unit. The treatment was considered a fixed effect and the measurement year and block were considered random effects. We used the Palmer Drought Severity Index (PDSI, a measure of drought) for Climate Division 3, Georgia from January, 2014 to December, 2105 to determine the level of drought in each month (http://www.ncdc.noaa.gov).

Results

Seasonal pattern of E_S, E_H, E_R and E_M

Fertilization significantly decreased E_{S} and E_{R} but not E_{H} or E_{M} in the two-year measurement period and in the growing season (Table 3.2, Table 3.3). There was a 22% reduction in E_{S} and a 19 % reduction in E_{R} in F treatments in 2014 to 2015, respectively. There was no significant effect of R or FR on E_{S}, E_{R}, E_{H}, and E_{M} (Table 3.3). In the dormant season, the there was a significant interaction between R and date and between FR and date for E_{S}. There was also an interaction between F and date for E_{H}. Soil moisture was significantly reduced by R and FR in untrenched E_{S} plots, and in the trenched E_{H} and E_{M} subplots during the study period as a whole and in the growing season (Table 3.3; Figure S3.3, Figure S3.4). Throughfall reduction significantly decreased soil moisture by 20%. There was no treatment effect on soil temperature, although soil temperature did differ significantly with measurement date (Table 3.3, Figure S3.2). The apparent Q_{10} of E_{S}, E_{H} and E_{R} in the dormant season was significantly higher than in the growing season (Table 3.2).

There were strong seasonal patterns in E_S and E_H in the four treatments over the measurement period (Figure 3.1). However, there was less seasonal variation in E_R and no seasonal variation in E_M among the treatments. Over the study period, E_H ranged from 0.45 to 2.46 µmol CO_2 m^{-2} s^{-1} in C, from 0.61 to 2.00 µmol CO_2 m^{-2} s^{-1} in F, from 0.52 to 2.11 µmol CO_2 m^{-2} s^{-1} in FR and from 0.45 to 2.06 µmol CO_2 m^{-2} s^{-1} in R treatments. Root CO_2 efflux varied between 0.32 and 1.18 µmol CO_2 $m^{-2}s^{-1}$ in C, between 0.14 and 0.80 µmol CO_2 m^{-2} s^{-1} in F, between 0.21 and 1.02 µmol CO_2 m^{-2} s^{-1} in FR and between 0.27 and 1.22 µmol CO_2 m^{-2} s^{-1} in R. Compared to E_H and E_R, E_M values were relatively low and the contribution of E_M to E_S was less than 15% in all treatments. In 2015, when water availability changed from moderate drought (July) to no drought (August) based on the Palmer Drought Index, significant increases in E_S, E_H and E_R were observed: E_S increased by 30%, E_H by 33% and E_R by 40%.

Effects of soil temperature and moisture on E_S, E_H, E_R and E_M

The relationships between E_H, E_R or E_M and soil temperature and moisture depended on the measurement period (Table 3.4). Across the total measurement period, between 45% and 64% of the variation in E_S was explained by soil temperature, soil moisture and their interaction. The R^2 for E_S was higher in the dormant season than in the growing season, especially in the control treatment. Soil temperature, moisture and their interaction accounted for between 47% and 56% of the variation in E_H. The R^2 for E_H was low (0.32 to 0.37) in all treatments in the growing season, compared with the total measurement period. Over the total measurement period, between 20 % and 34 % of the variation in E_R was explained by soil temperature, soil moisture and their interaction. There were no significant relationships between E_R and those factors in the dormant season in all treatments. No correlations were found between E_R and the factors in other treatments, except F in the growing season. Root CO_2 efflux values were less dependent on soil temperature and moisture, except in F across the total measurement period. Ectomycorrhizal hyphae CO_2 efflux was independent of soil temperature, moisture and their interaction in the C and F treatments and only weakly correlated in the FR and R treatments. In the FR and R treatments, only 9 to 11% percent of variation in E_M could be explained by soil temperature and moisture.

Soil temperature explained more variation in E_S, E_H and E_R in non-drought periods compared to drought periods, with the exception of E_H in C (Table 3.5). Apparent Q_{10} of E_S, E_H and E_R was slightly, but not statistically significantly, higher in the non-drought period than drought period with the exception of the Q_{10} of E_H in the C treatment. The E_{S15} (E_S normalized to 15°C) generally increased with increasing W in C, F and FR treatments, although E_{S15} declined after W reached a threshold at about 20% in C (Figure 3.2). Heterotrophic CO_2 efflux normalized at 15 °C (E_{H15}) also increased with increasing W in C and F, but not in FR and R.

Relationship between LAI and E_H and E_{RC} /E_{SC}

Leaf area index varied with measurement date (Figure S 3.5). The highest *LAI* was in the F treatment and the lowest was in the R treatment. Fertilization increased *LAI* by 27% during the measurement period, compared to C. There was no difference in *LAI* between the R, FR and C treatments. There were positively linear relationships between *LAI* and E_H in the C, FR and R treatments (Figure 3.3). There were also weak positive correlations between *LAI* and E_S and E_R ($p > 0.05$). Leaf area index explained more than 50% of the variation in E_H in the FR treatment. However, *LAI* was negatively correlated with the ratio of monthly cumulative E_R to monthly cumulative E_S (E_{RC} /E_{SC}) in all treatments (Figure 3.4). Over the study period, the contribution of E_R to E_S ranged from 24% to 45% in C, 21% to 41% in F, 27% to 54% in FR and 32% to 47% in R. Moreover, E_{RC} /E_{SC} was highest in the dormant season and lowest in the growing season.

E_H, E_R and E_M

The contribution of E_H, E_R and E_M to E_S changed between the growing season and dormant season (Figure 3.5). Over the entire growing season, the cumulative contribution E_H to cumulative E_S ranged from 51% to 61% in the four treatments. In the dormant season, the cumulative contribution of E_H to E_S was lower, ranging from 42 to 45%. In contrast, the cumulative contribution of E_R to Es was higher in the dormant season (between 38% and 48%) than in the growing season (between 29% and 40%). Cumulative E_M contributed about 10% to cumulative E_S in the growing season in all treatments. In the dormant season, the contribution of cumulative E_M to cumulative E_S was also about 10%, except in the F treatment, where it was higher (17 %).

Fertilization significantly reduced annual cumulative E_R but it had no effect on E_H or E_M (Figure 3.6; Table 3.6). There was no effect of R or FR on annual E_H, E_R and E_M. There was an interaction between F and year on E_R. The average annual E_H was 389 g C m^{-2} yr^{-1} for C, 346 g C m^{-2} yr^{-1} for FR, 331 g C m^{-2} yr^{-1} in F and 325 g C m^{-2} yr^{-1} for R, respectively. The average annual E_R was 244 g C m^{-2} yr^{-1} in R, 238 g C m^{-2} yr^{-1} in FR, 234 g C m^{-2} yr^{-1} in C and 166 g C m^{-2} yr^{-1} in F, respectively. Annual cumulative E_M ranged from 78 g C m^{-2} yr^{-1} (R) to 64 (FR). The largest contribution to annual cumulative E_S was cumulative E_H, ranging from 58% (F) to 50% (R). Annual cumulative E_R accounted for 29 to 37% of E_S and annual cumulative E_M accounted for less than 12% in all treatments. No relationships were detected between annual cumulative E_S, E_H and E_R and litterfall or foliage production. There were positive relationships between E_R normalized at 15$^\mathrm{o}$C (E_R15) and stem CO_2 efflux normalized at 15$^\mathrm{o}$C (E_STEM15) in the four treatments during the total measurement period (Table S3.1; Figure 3.7).

Discussion

Compared with other studies that have quantified the contribution of E_H, E_R and E_M to E_S, the values in our study were in the middle of those reported for other temperate forests (Table 3.7). The dominant component of E_S was E_H (50% to 58%) which was similar to the findings in other studies in loblolly pine plantations (Templeton et al., 2015; Maier and Kress 2000, Noormets, 2010), and consistent with the 30% to 70% range reported for the contribution of E_H to E_S in temperate forest ecosystems (Bond-Lamberty et al., 2004). The contribution of E_A (E_R + E_M) to E_S ranged from 42% to 50% in the different treatments, which was similar to the average of 55% reported for other temperate coniferous forests (Subke et al., 2006). The annual contribution of E_M to E_S ranged from 10% to 12% and was in the lower end of the values reported by other studies (8% to 44%) in boreal and temperate forests.

Effect of fertilization on the components of E_S: E_R, E_H and E_M

Root CO_2 efflux, but not E_H, was reduced by fertilization, which partly supported our first hypothesis. Consistent with our results, there was a decrease in E_A in a 7-year-old loblolly pine plantation after seven years of fertilization, and in a 31-year-old red pine (*Pinus resinosa*) plantation after four years of fertilization (Haynes and Gower, 1995; Samuelson et al., 2009). However, the response of E_A to fertilization varies widely. Fertilization was reported to increase E_A in 1-year-old pot-grown loblolly seedlings with short-term (<1 year) fertilization and in a 33-year old loblolly pine plantation with long-term (>20 year) fertilization (Gough and Seiler, 2004; Tyree et al., 2006). Others reported that there was no effect of fertilization on E_A or E_R in an 11-year-old loblolly pine plantation after four year of nutrient addition and in a 25-year-old loblolly pine plantation after receiving one year of nitrogen fertilizer (Maier and Kress, 2000; Drake et al., 2008).

The possible causes of decreased E_R or E_A after fertilization include: (1) reduction in fine root biomass (Haynes and Gower, 1995; Olsson et al., 2005; Phillips and Fahey, 2009; Samuelson et al., 2009; Burton et al., 2012); (2) a shift of production from fine roots to coarse roots (Maier and Kress, 2000; Retzlaff et al., 2001); (3) a change in fine root tissue chemistry (Drake et al. 2008); (4) the upward transport of a portion of CO_2 derived from E_R through sapflow (Aubrey and Teskey, 2009; Yang et al., 2012; Yang et al., 2016); and (5) suppression of the production of mycorrhizal fungi (Nilsson and Wallander, 2003;Wallander et al., 2011; Hasselquist et al., 2012; Pritchard et al., 2014). In addition, the effect of fertilization on E_R or E_A in forests appears to depend on stand age, forest type, fertilizer amount and experimental duration (Liu and Greaver, 2010; Lu et al., 2011; Zhou et al., 2014).

Fertilization often results in a reduction in fine root biomass in trees (Haynes and Gower, 1995; Samuelson et al., 2009) but since we did not directly measure the fine root biomass in our experiments, we cannot attribute the reduction in E_R and E_S we observed specifically to that cause. In addition, other root morphological and physiological traits related to nutrient acquisition may play role in the reduction in E_R. Specific root length, tissue density, root order, nitrogen and non-structural carbon content have been shown to affect E_R (King et al., 2002; George et al., 2003; Drake et al., 2008; Phillips et al., 2012; Taylor et al., 2014). Retzlaff et al. (2001) reported that coarse root production was favored over fine root production in a 5-year-old loblolly pine plantation after fertilization. The trend of increased coarse root production after fertilization was also found in our study (Yang, unpublished data). Since coarse roots of loblolly pine have lower rates of respiration than fine roots (Maier and Kress, 2000; Maier et al., 2004) the increased coarse root production in our study is a possible explanation for the reduced E_S and E_R in the fertilizer treatment.

A change of fine root chemistry was likely not an important factor in the reduction of E_S and E_R because Drake et al. (2008) reported that nitrogen fertilization of a loblolly pine stand had no direct effect on fine root carbohydrates. They also reported that fertilization increased fine root nitrogen content but did not increase fine root respiration.

There is increasing evidence that a portion of the CO_2-derived from root respiration can be transported aboveground via the xylem stream and lead to an underestimation of E_A (Aubrey and Teskey, 2009; Angert et al., 2012; Yang et al., 2012; Bloemen et al., 2013; Yang et al., 2016). However, some authors have not observed that relationship in conifers (Maier and Clinton, 2006; Ubierna et al., 2009). These correlations indirectly suggest that the upward transport of CO_2 from roots may be causing an underestimation of E_R. However, we observed no significant relationships between E_A (E_R+ E_M) normalized at 15ºC and E_{STEM15}. Maier and Clinton (2006) reported xylem transport of CO_2 had no direct influence of stem CO_2 efflux in young loblolly pine trees and concluded that stem CO_2 efflux was uncoupled from internal xylem CO_2 flux in loblolly pine. Additionally, using analysis of covariance, there were no significant differences detected between the slopes of the correlations between E_{R15} and E_{STEM15} in the four treatments. This implies that other causes are more likely than xylem CO_2 transport to explain the reduction in E_R in the fertilization treatment.

The suppression of the distribution and production of mycorrhizal fungi caused by fertilization has been reported in the boreal and temperate forests (Nilsson and Wallander, 2003; Wallander et al., 2011; Hasselquist et al., 2012; Pritchard et al., 2014). In loblolly pine plantations after 6 years of fertilization, a decline in ectomycorrhizal root tip production was reported (Pritchard et al., 2014). However, Taylor et al. (2014) found the opposite trend in root tip production at the same study site and over the same time period. The conflicting results in these studies may be attributable to different measurement methods. However, the lack of response of E_M to fertilization on in our study implies that reduction of mycorrhizal fungi was probably not the cause of reduced E_S or E_A in the fertilization treatment. However, we did not measure fine root biomass, mycorrhizal production, and fine root chemistry, so the exact reason for the reduction in E_S and E_R in the fertilization treatment cannot be determined. However, we think it is likely that the shift of root production from fine root to coarse roots may be important factors contributing to decreased E_S and E_R in fertilization treatments.

There was a trend of lower E_H in the fertilization treatment compared to the other treatments, but the difference (14%) was not statistically significant. This trend was consistent with observations from other studies. Using a meta-analysis of 36 nitrogen–manipulation studies in forest ecosystems, Janssens et al. (2010) reported that E_H declined on average by 15% in response to nitrogen amendments. Similarly, Zhou et al. (2014) reported that there was a mean reduction in E_H by 12% after fertilization in 101 temperate forests. Possible reasons for the negative effect of fertilization on E_H include: (1) a reduction in microbial biomass (Treseder, 2008; Janssens et al., 2010; Zhou et al., 2014); (2) a decline in quantity and quality of root exudates which can affect saprotrophic organism activity and decomposition (priming effect)(Henry et al., 2005; Lagomarsino et al., 2006; Janssens et al., 2010); (3) a change of critical gene expression for microbial decomposition, which can cause shifts in the microbial community and decomposing-enzymes (Edwards et al., 2011; Gallo et al., 2004; Zak et al., 2011; Zhou et al., 2014); and (4) a reduction in litter decomposition after nitrogen amendments in species with low quality litter (e.g. *Pinus, Picea, Fagus, Quercus*) in temperate forests (Janssens et al., 2010; Zhou et al., 2014).

There was no effect of fertilization on E_M in our study. This result was consistent with the study of Hendricks et al. (2015) who reported that fertilization did not significantly change ectomycorrhizal hyphae dynamics in a 25-year-old longleaf pine plantation. A possible explanation may be that the absolute amount of carbon allocated belowground was comparable to the control even though fertilization increased above-ground production and decreased belowground carbon allocation. Other explanations could be that quantity of the ectomycorrhizal hyphae or their growth dynamics did not change (Clemmensen et al., 2015, Hendricks et al., 2015). In other coniferous forests fertilization has been reported to decrease E_M by reducing colonization or through ectomycorrhizal fungal malfunction (Hasselqusit et al., 2012; Vallack et al., 2012). However, previous studies in loblolly pine have provided conflicting evidence for this. Fertilization was reported to have caused a decline in ecotomycorrhizal root tip production after 6 years of nitrogen fertilization in loblolly pine plantations (Pritchard et al., 2014). However, Taylor et al., (2014) found the opposite trend of the ectomycorrhizal root tip production at the same site.

Effect of throughfall reduction on the components of E_S: E_R, E_H and E_M

Our results were not consistent with our second hypothesis that throughfall reduction would reduce E_R. There was no effect of throughfall reduction in any of the CO_2 fluxes we measured. These results are consistent with the conclusion from a meta-analysis of studies from thirteen temperate forests that decreased precipitation had no effect on E_S, E_A and E_H (Liu et al., 2016). In other studies, there have been many reports of drought reducing E_S. Drought has decreased E_H (Scott-Denton, 2006; Borken et al., 2006; Noormets et al., 2010; Suseela et al., 2012), decreased E_A (Risk et al., 2012; Hinko-Najera et al., 2015), decreased both E_A and E_H (Schindlbacher et al. 2012), and decreased E_M (Heinemeyer et al., 2007). However, the effect of drought on E_S, E_H and E_R depends on drought severity and duration, and the variation in the seasonal pattern of rainfall distribution and in interannual precipitation (Shi et al., 2014; Hinko-Najera et al., 2015). The 30% reduction in throughfall reduction in this study can be classified as a moderate drought (25% to 40% decrease in annual precipitation), which in other studies had little effect on E_S or E_R (Shi et al., 2014; Liu et al., 2016).

Several factors may have contributed to a lack of response to the throughfall reduction treatment. In the middle of the study, there was a year with abnormally high precipitation (304 mm above average). In addition, relatively high rainfall occurred in two of the dormant seasons (40% and 47% to total annual precipitation) during the study. This would have increased soil water storage, and buffered the effect of the throughfall reduction treatment during the growing season. Also, based on the monthly PDSI, during the study there was only two periods of drought in the region, a mild drought period from February, 2014 to June, 2015 and a moderate drought in July, 2015. Finally, deep root water uptake and hydraulic redistribution of water from moist to dry soils via loblolly pine roots may have compensated for the reduction in throughfall . Even for loblolly pines growing in clay-loam soils there is measurable hydraulic redistribution (Domec et al., 2010; Domec et al., 2012).

The lack of response to the throughfall reduction treatment does not mean that soil moisture had no effect on E_S, E_R or E_H. Soil moisture can limit soil biological activity at both high and low moisture contents. After controlling for temperature a relationship between soil moisture and E_{S15} or E_{H15} was observed in the control and fertilization treatments. However, it was interesting that this pattern was not detected in the throughfall reduction treatment. For the study as a whole, there were additional indications that soil moisture was influencing E_S to some extent. For example, soil temperature explained more variation in E_S, E_H and E_R in the wet periods than in the drought periods. The significant increase in E_S, E_H and E_R from July (a period of moderate drought) to August (a period of no drought) in 2015 also indicated that E_S, E_H and E_R were affected by soil moisture.

Relationships between LAI and E_H and E_{RC}/ E_{SC}

The positive relationship between E_H and *LAI* and the negative relationship between E_{RC}/ E_{SC} and *LAI* partially supported our third hypothesis. A close correlation between *GPP* and E_S, E_A and E_H has been reported in different forest ecosystems (Litton et al., 2007; Bond-Lamberty et al., 2004; Hopkins et al., 2012; Chen et al., 2014). However, we did not observe a positive correlation between E_R and *LAI* in this study. In a number of studies it has been demonstrated that canopy photosynthesis can affect E_S by affecting E_R or E_A (Subke et al., 2006; Bahn et al., 2008; Subke et al., 2012; Hopkins et al., 2012). Leaf area index, which is a proxy of GPP, has been correlated with E_S and E_H in forest ecosystems (Lindroth et al., 2008; Reichstein et al.,2003; Yustie et al., 2004; Hibbard et al., 2005; Oishi et al., 2013). Reichstein et al. (2003) showed that E_S was positively, and linearly, correlated with peak *LAI* in 17 forest and shrubland sites. However, there are exceptions. Palmroth et al. (2006) found an inverse relationship between E_S and *LAI* in several temperate forests. Ngao et al., (2012) also found that stands with a higher *LAI* had a lower E_{10} (E_S normalized at 10^{o}C), which was due to increased soil water deficit caused by greater transpiration and precipitation interception.

Conclusions

Fertilization reduced E_S and E_R while the 30% throughfall reduction treatment had no effect on E_H, E_R and E_M. We speculate that the decrease in E_R may have been related to a shift in production from fine roots to coarse roots and to the long lifespan of mycorrhizal fine roots. Soil temperature, moisture and their interaction were good predictors of E_S and E_H, but they explained less variation in E_R and E_M in four treatments. Soil temperature explained more variation in E_S, E_H and E_R than soil moisture, especially in non-drought periods compared to drought periods. The correlation between E_H and *LAI* indicated that there would like be a close correlation between *GPP* and E_S. Among the treatments, the contribution of E_H to E_S ranged from 50% to 58% and the contribution of E_M to E_S ranged from 10% to 12%. Although E_M was the smallest component flux, we suggest that more estimates of it in temperate forests will improve our understanding of E_R and its contribution to E_S. The relatively larger effect of temperature on E_S, and its component fluxes, compared with soil moisture, and the lack of response generally to the throughfall reduction treatment, suggest that increasing temperatures in the future may increase E_S and its component fluxes in loblolly pine plantations, although the potential interactions between temperature, transpiration, *LAI* and soil water availability on those fluxes make it very hard to predict the outcome.

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Table 3.1. Site mean annual temperature (MAT) and annual precipitation (AP) in control plots from 2013 to 2015. The symbol "ΔP" indicates the difference in precipitation between the measurement year and the 30-year average annual precipitation (1983-2012). The growth characteristics includes diameter at breast height (*DBH*) and leaf area index (*LAI*).

Year	Age (year)	MAT (^oC)	AP (mm)	ΔP (mm)	Stand density (tree ha^{-1})	Basal Area ($m^{-2}ha^{-1}$)	DBH (cm)	LAI (m^2m^{-2})	Height (m)
2013	8	18.2	1413	+304	1373	16.13	12.12	2.7	8.8
2014	9	17.9	989	-120	1373	19.78	13.44	3.0	10.1
2015	10	17.9	1276	+167	1373			3.5	

Table 3.2. Overall means of soil CO_2 efflux (E_S), heterotrophic CO_2 efflux (E_H), root CO_2 efflux (E_R) and ectomycorrhizal hyphae CO_2 efflux (E_M), apparent temperature sensitivity (Q_{10}) of E_S, E_H, E_R and E_M during the two-year measurement period (Total), in the growing season (GS) and the dormant season (DS). The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only. The abbreviation of Trts represents treatments. In each treatment, there were significant differences in Q_{10} of E_S, E_H, and E_R between the growing season and the dormant season ($p<0.05$).

Period	Trts	E_S	E_H	E_R	E_M	Q_{10}			
		(μmol CO_2 m^{-2} s^{-1})				E_S	E_H	E_R	E_M
Total	C	2.09a (0.19)	1.60 (0.15)	0.59a (0.05)	0.22 (0.05)	2.00 (0.19)	2.22 (0.37)	1.54 (0.19)	1.56 (0.44)
	F	1.62b (0.15)	1.39 (0.13)	0.48b (0.05)	0.25 (0.05)	2.22 (0.05)	2.13 (0.28)	2.15 (0.57)	1.77 (0.64)
	FR	1.88ab (0.14)	1.38 (0.13)	0.59a (0.05)	0.15 (0.17)	2.06 (0.16)	2.29 (0.09)	1.83 (0.22)	1.69 (0.53)
	R	1.97a (0.17)	1.29 (0.12)	0.63a (0.06)	0.25 (0.08)	2.27 (0.17)	2.43 (0.58)	1.88 (0.41)	2.25 (0.82)
GS	C	2.38 a(0.19)	1.84 (0.14)	0.69 (0.06)	0.23 (0.07)	1.83 (0.56)	1.85 (0.16)	1.40 (0.90)	
	F	1.84 b(0.15)	1.53 (0.14)	0.56 (0.05)	0.29 (0.13)	1.54 (0.29)	1.99 (0.14)	1.29 (0.28)	
	FR	1.98 ab(0.13)	1.53 (0.14)	0.62 (0.05)	0.16 (0.06)	1.20 (0.42)	1.96 (0.38)	1.89 (0.33)	
	R	2.23 a(0.14)	1.43 (0.12)	0.81 (0.05)	0.27 (0.09)	1.54 (0.36)	1.90 (0.24)	1.12 (0.62)	
DS	C	1.29a (0.18)	1.02 (0.16)	0.47 (0.06)	0.20 (0.08)	3.18 (1.22)	4.67 (2.34)	3.16 (2.66)	
	F	1.01ab (0.17)	1.02 (0.20)	0.28 (0.06)	0.18 (0.31)	5.00 (2.10)	6.16 (1.50)	5.64 (5.06)	
	FR	1.24a (0.22)	0.96 (0.23)	0.39 (0.09)	0.09 (0.10)	4.79 (0.88)	5.98 (1.27)	4.53 (2.10)	
	R	1.25 a(0.25)	0.90 (0.19)	0.52 (0.07)	0.19 (0.07)	6.71 (1.77)	6.59 (1.69)	5.09 (3.70)	

Table 3.3. The *p*-values of treatment effects on soil $\mathrm{CO_2}$ efflux (E_{S}), heterotrophic $\mathrm{CO_2}$ efflux (E_{H}), root $\mathrm{CO_2}$ efflux (E_{R}) and ectomycorrhizal hyphae $\mathrm{CO_2}$ efflux (E_{M}). The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only. A non-significant treatment effect is represented by ns.

Period		Treatments						
		F	R	FR	Date	F × Date	R× Date	FR×date
Total	T	ns	ns	ns	<0.01	ns	ns	ns
	W	ns	<0.005	<0.005	ns	ns	ns	ns
	E_{S}	<0.001	ns	ns	<0.001	ns	ns	ns
	E_{H}	ns	ns	ns	ns	ns	ns	ns
	E_{R}	<0.01	ns	ns	<0.001	ns	ns	ns
	E_{M}	ns	ns	ns	ns	ns	ns	ns
GS	T	ns	ns	ns	<0.05	ns	ns	ns
	W	ns	<0.01	<0.001	ns	ns	ns	ns
	E_{S}	<0.001	ns	ns	<0.05	ns	ns	ns
	E_{H}	ns	ns	ns	ns	ns	ns	ns
	E_{R}	<0.01	ns	ns	<0.01	ns	ns	ns
	E_{M}	ns	ns	ns	Ns	ns	ns	ns
DS	T	ns	<0.1	ns	<0.05	ns	ns	ns
	W	ns	ns	ns	ns	ns	ns	ns
	E_{S}	ns	ns	ns	ns	ns	<0.01	<0.05
	E_{H}	ns	ns	ns	ns	<0.05	ns	ns
	E_{R}	ns	ns	ns	ns	ns	ns	ns
	E_{M}	ns	ns	ns	ns	ns	ns	ns

Table 3.4. Regression models of soil CO_2 efflux (E_S) (μmol CO_2 m^{-2} s^{-1}) heterotrophic CO_2 efflux (E_H), root CO_2 efflux (E_R) and ectomycorrhizal hyphae CO_2 efflux (E_M) predicted using soil temperature at 10cm soil depth (T, °C) and soil volumetric water content at 20cm depth (W, %) in the four treatments in the two-year measurement period (Total), as well as in the growing season and the dormant season. Dormant season (DS) was from November to February in the following year. Growing season (GS) was from March to October. The regression models are of form: Ln (E_S) = a + bT + c W+ d $T \times W$, where Ln is natural logarithm. The lowercase letters a, b, c and d are regression coefficients. The letter n is sample size. Adj-R^2 is the adjusted determination coefficient. RMSE stands for root mean square error. AICc is the Akaike information criterion corrected for finite sample size to account for overfitting with small sample size. The *p*-value is the significance level associated with the F-statistics. The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

Efflux	Period	Treatments	a	b	c	d	n	F	Adj-R^2	RMSE	*p*	AICc
E_S	Total	C	1.28	-0.0197	-0.0653	0.00307	56	35.78	0.64	0.24	<0.001	9.59
		F	-0.0692	0.01425	-0.0394	0.00304	56	24.26	0.54	0.27	<0.001	27.14
		FR	0.785	-0.0155	-0.0645	0.004	56	16.96	0.45	0.28	<0.001	27.58
		R	1.018	-0.012	-0.076	0.0037	56	31.1	0.61	0.24	<0.001	11.6
	DS	C	0.389	0.06	-0.03		13	15.39	0.66	0.18	<0.001	-1.35
		F	-1.26	0.10			14	7.99	0.32	0.25	0.01	7.56

		FR	0.23	0.06	-0.037		13	6.62	0.43	0.26	0.01	10.9
		R	-0.57	0.12	-0.03		13	6.19	0.41	0.29	0.01	13.6
	GS	C	1.48	-0.029	-0.068	0.003	40	5.204	0.23	0.25	<0.005	13.3
		F	0.138	0.0077	-0.034	0.0026	40	5.144	0.22	0.28	<0.005	22.9
		FR	0.19	0.022			42	3.71	0.18	0.28	0.04	18.29
		R	0.52	0.019	-0.01		41	6.157	0.19	0.2	<0.005	-8.37
E_{H}	Total	C	-0.53	0.048	-0.008	0.0006	56	21.23	0.51	0.31	<0.001	41.67
		F	0.404	-0.0091	-0.069	0.0044	56	21.39	0.51	0.33	<0.001	49.8
		FR	1.17	-0.033	-0.104	0.005	56	25.5	0.56	0.29	<0.001	34.14
		R	-0.06	0.04	-0.02		57	26.79	0.47	0.34	<0.001	48.6
	DS	FR	7.85	-0.6	-0.419	0.03	12	6.19	0.51	0.28	<0.01	15.22
		R	-1.44	0.12			14	5.255	0.22	0.37	0.03	20.1
	GS	C	-0.56	0.054			42	26.04	0.37	0.25	<0.001	11.33
		F	0.098	0.003	-0.055	0.0039	40	8.1	0.33	0.3	<0.001	31.16
		FR	0.54	-0.0047	-0.07	0.0038	40	8.42	0.34	0.28	<0.001	23.85
		R	0.27	0.027	-0.023		41	10.89	0.32	0.31	<0.001	31.45
E_{R}	Total	C	0.29	-0.040	-0.055	0.003	56	6.43	0.22	0.3	<0.001	39.6
		F	-0.74	-0.034	-0.07	0.006	56	24.55	0.34	0.28	<0.001	29.8
		FR	-0.39	-0.03	-0.06	0.005	56	5.91	0.2	0.37	<0.01	63.48
		R	0.62	-0.05	-0.09	0.005	56	9.53	0.3	0.29	<0.001	34.04
	GS	F	-1.14	-0.016	-0.04	0.004	40	10.54	0.4	0.27	<0.001	21.05
E_{M}	Total	FR	-3.37	0.074	0.047		51	3.45	0.09	0.72	0.03	126.8
		R	0.28	-0.08	-0.12	0.007	53	3.38	0.11	0.6	0.02	122.3

Table 3.5. Regression models between soil CO_2 efflux (E_S), heterotrophic CO_2 efflux (E_H) and root CO_2 efflux (E_R) and soil temperature at 10cm soil depth (T) in drought and non-drought periods. The letter n indicates sample size, Adj R^2 indicates adjusted coefficient of determination. The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

Efflux	Treatments	Drought	Equations	n	Adj R^2	p	Q_{10}
E_S	C	Yes	$E_S = 0.957*\exp(0.042*T)$	44	0.311	<0.0001	1.52
		No	$E_S = 0.787*\exp(0.049*T)$	16	0.608	0.0002	1.63
	F	Yes	$E_S = 0.716*\exp(0.043*T)$	44	0.304	<0.0001	1.54
		No	$E_S = 0.414*\exp(0.079*T)$	16	0.817	<0.0001	2.20
	FR	Yes	$E_S = 0.832*\exp(0.039*T)$	44	0.308	<0.0001	1.48
		No	$E_S = 0.750*\exp(0.053*T)$	16	0.563	0.0008	1.70
	R	Yes	$E_S = 0.937*\exp(0.039*T)$	44	0.375	<0.0001	1.49
		No	$E_S = 0.755*\exp(0.054*T)$	16	0.615	0.0002	1.72
E_H	C	Yes	$E_S = 0.535*\exp(0.058*T)$	44	0.526	<0.0001	1.78
		No	$E_S = 0.672*\exp(0.054*T)$	16	0.419	0.007	1.71
	F	Yes	$E_S = 0.554*\exp(0.055*T)$	44	0.337	<0.0001	1.73
		No	$E_S = 0.518*\exp(0.057*T)$	16	0.557	0.0009	1.77
	FR	Yes	$E_S = 0.562*\exp(0.050*T)$	44	0.410	<0.0001	1.65
		No	$E_S = 0.566*\exp(0.065*T)$	16	0.478	0.003	1.92
	R	Yes	$E_S = 0.600*\exp(0.047*T)$	44	0.383	<0.0001	1.59
		No	$E_S = 0.650*\exp(0.052*T)$	16	0.398	0.009	1.68
E_R	C	Yes	$E_S = 0.251 *\exp(0.045*T)$	44	0.101	0.035	1.57
		No	$E_S = 0.172*\exp(0.053*T)$	16	0.241	0.044	1.69
	F	Yes	$E_S = 0.162*\exp(0.054*T)$	44	0.149	0.009	1.72
		No	$E_S = 0.063*\exp(0.115*T)$	16	0.604	0.0004	2.16
	FR	Yes	$E_S = 0.210*\exp(0.055*T)$	44	0. 265	0.0004	1.73
		No	$E_S = 0.141*\exp(0.083*T)$	16	0.645	0.0001	2.29
	R	Yes	$E_S = 0.422*\exp(0.032*T)$	44	0.247	0.003	1.38
		No	$E_S = 0.254*\exp(0.042*T)$	16	0.313	0.0243	1.52

Table 3.6. The *p*-values of treatment effects on annual soil CO_2 efflux (E_S), heterotrophic CO_2 efflux (E_H), root CO_2 efflux (E_R), ectomycorrhizal hyphae CO_2 efflux (E_M). The abbreviations of the treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only. No significant treatment effect is indicated by ns. All carbon flux unit is g C m^{-2} yr^{-1}.

Treatment	Annual E_S	AnnualE_H	Annual E_R	Annual E_M
F	<.001	0.067	<.001	ns
R	ns	ns	ns	ns
FR	ns	ns	ns	ns
Year	ns	ns	0.051	ns
F × Year	ns	ns	0.047	ns
R × Year	ns	ns	ns	ns
FR × Year	ns	ns	ns	ns

Table. 3.7. The annual soil CO_2 efflux (E_S, g C m^{-2} yr^{-1}), the contribution of heterotrophic CO_2 efflux (E_H), root CO_2 efflux (E_R), ectomycorrhizal hyphae CO_2 efflux (E_M) to the annual E_S in different forest ecosystems with ectomycorrhizal colonization. All the CO_2 efflux measurement was made with an Infrared Gas Analyzer (IRGA) except Vallack et al. (2012) who used isotopic pulse labelling. In the Biome column, T represents temperate forests and B represents boreal forests. In the Method column, the small letter a represents mesh bags and b represents tubes with mesh window and c represents rhizomorphic mats.

References	MAT (ºC)	MAP (mm)	Biome	Species	Age (year)	Treatment	Treatment level	Method	E_S	E_R%	E_H%	E_M%
Andrew et al.,2014	18.3		T	*Populus tremuloides*		Ambient CO_2	Control	a				31
Andrew et al.,2014	18.3		T	*Populus tremuloides*		Elevated CO_2	+200ppm	a				31
Andrew et al.,2014	18.3		T	*Populus tremuloides*		Ambient O_3	33-67ppb	a				31
Andrew et al.,2014	18.3		T	*Populus tremuloides*		Elevated O_3	50-100ppb	a				31
Heineemeyer et al., 2007			T	*Pinus contorta*	15					15	65	25
Heinemeter et al., 2012b	9.4	780	T	*Quercus robur*	75-80				740	38	44	18
Hasselquist et al., 2012	1.2	520	B	*Pinus sylvestris*	70	Fertilization	Control	b		23	49	16
Hasselquist et al., 2012	1.2	520	B	*Pinus sylvestris*	70	Fertilization	20kg N $ha^{-1}yr^{-1}$	b		27	40	22
Hasselquist et al., 2012	1.2	520	B	*Pinus sylvestris*	70	Fertilization	100 kg N ha^{-1} yr^{-1}	b		19	53	8
Vallack et al., 2012	1.2	520	B	*Pinus sylvestris*		Fertilization	Control	b		48.9		

Vallack et al., 2012	1.2	520	B	*Pinus sylvestris*		Fertilization	100kg N ha^{-1} yr^{-1}	b		22.4		7
Matvienko et al., 2014		500	B	*Larix sibirica*				b	330			18
Matvienko et al., 2014		500	B	*Pinus sylvestris*				b	250			20
Moyano et al., 2008	8	750-800	T	*Fagus sylvatica*	1-250			a		44	53	3
Moyano et al., 2008	5.5	800-1015	T	*Picea abies*	50			a		45	47	8
Neumann& Matzner 2014	5.3	1156	B	*Picea abies*				a				18-44
Fenn et al., 2010	10.1	725.8	T	*Mixed deciduous species*				a	410	70	22	8
Phillips et al., 2012			T	*Pseudotsuga menziesii*	450			c				9

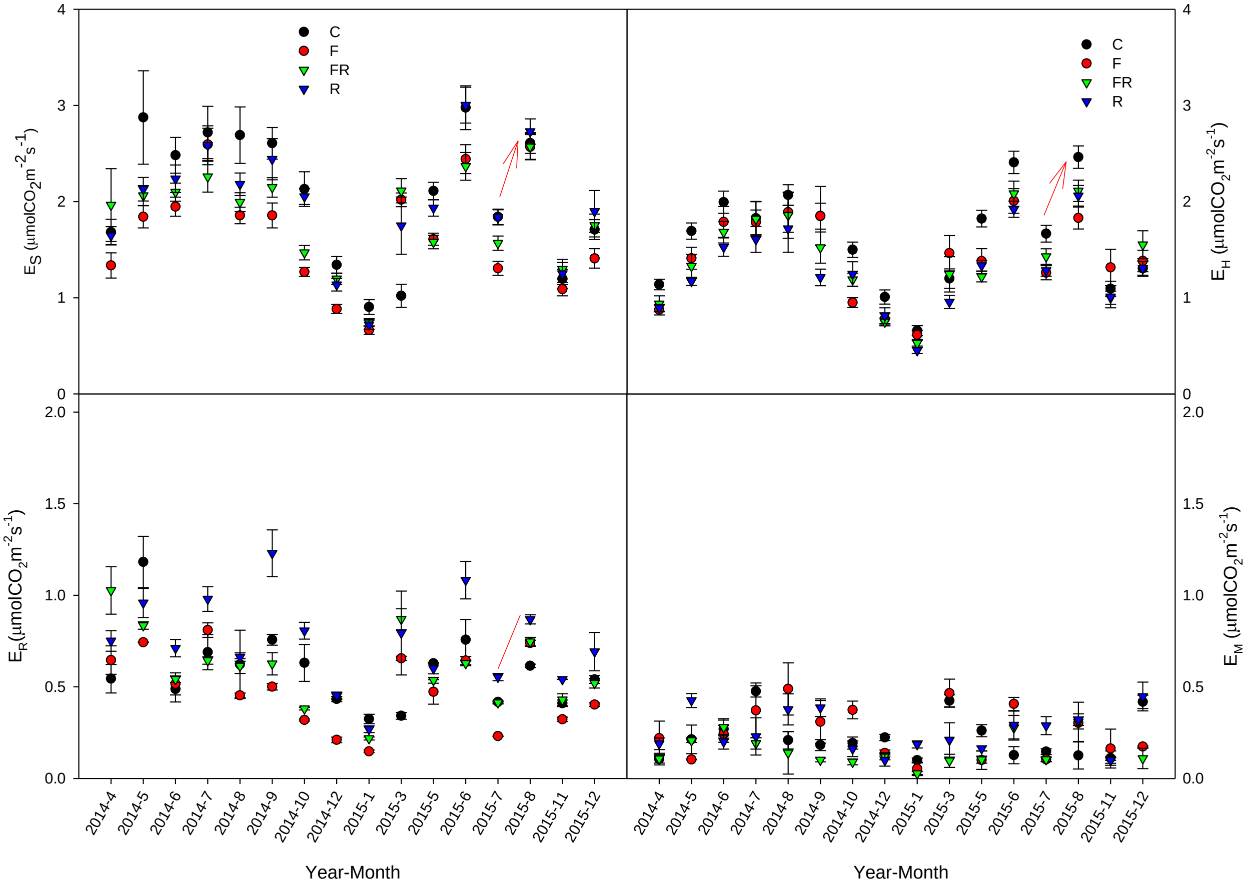

Figure 3.1. Seasonal patterns of soil CO_2 efflux (E_S), heterotrophic CO_2 efflux (E_H), root CO_2 efflux (E_R) and ectomycorrhizal hyphae CO_2 efflux (E_M) during the two-year measurement period. The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

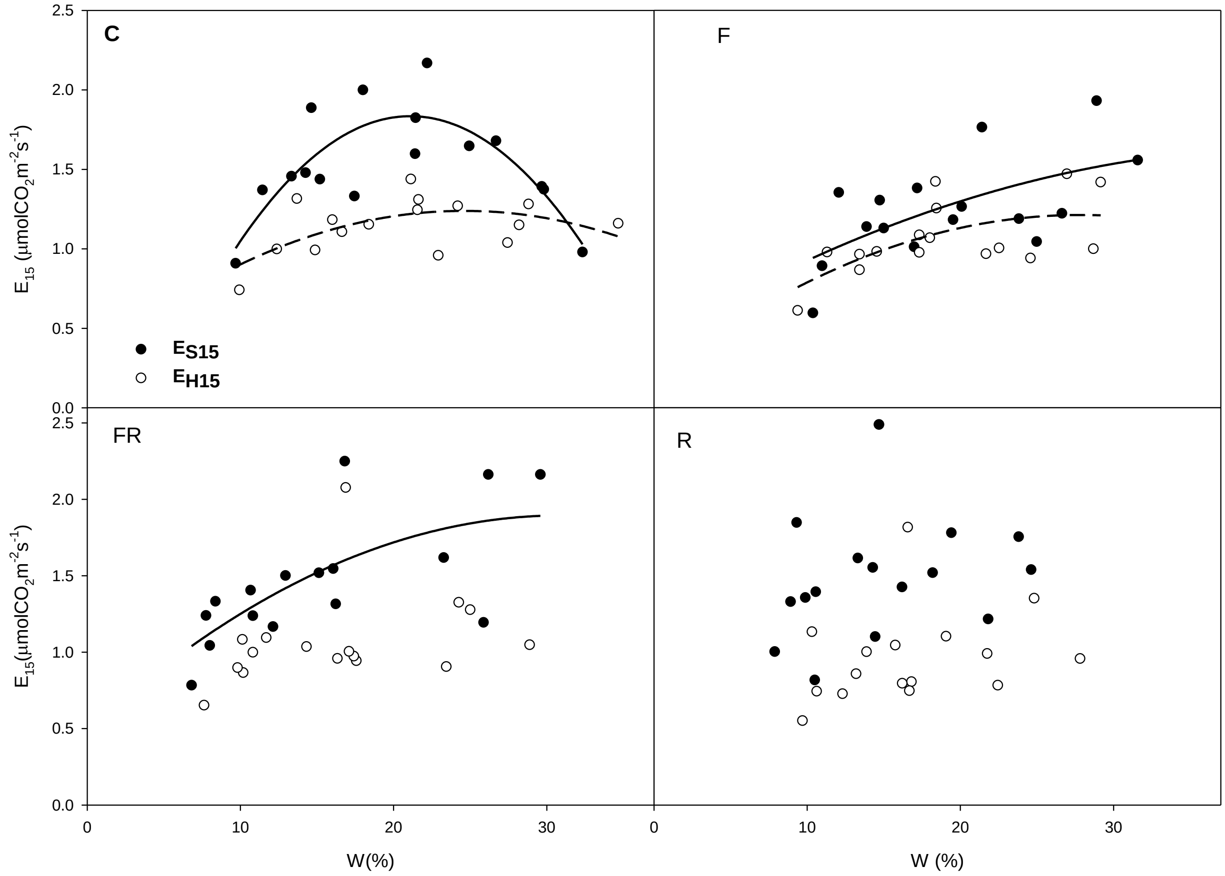

Figure 3.2. Relationships between soil CO_2 efflux normalized at 15°C (E_{S15}) and heterotrophic CO_2 efflux normalized at 15°C (E_{H15}) and soil moisture at 20cm depth (*W*). The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

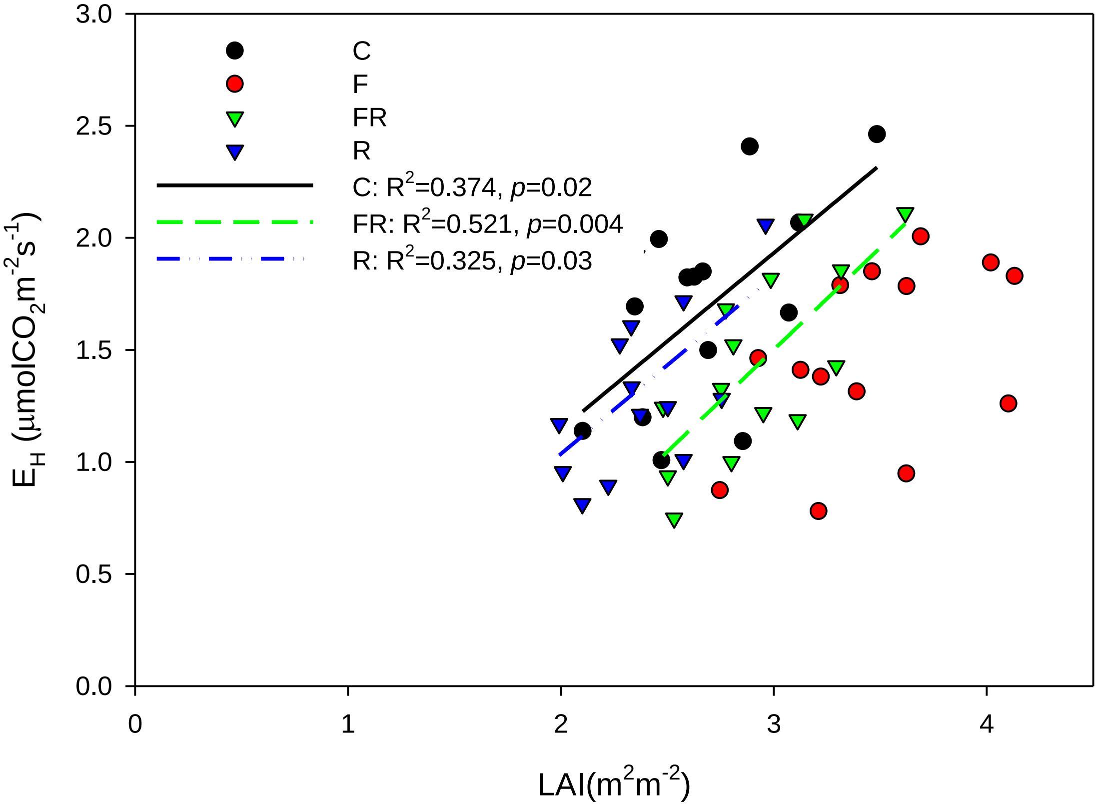

Figure 3.3. Relationships between heterotrophic CO_2 efflux (E_H) and leaf area index (*LAI*) in four treatments. The significant level was α=0.0001. The black solid line, the green dashed line and blue dashed-dotted line are the regression lines between E_H and *LAI* in the C, FR and R treatments, respectively. The abbreviations of the treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

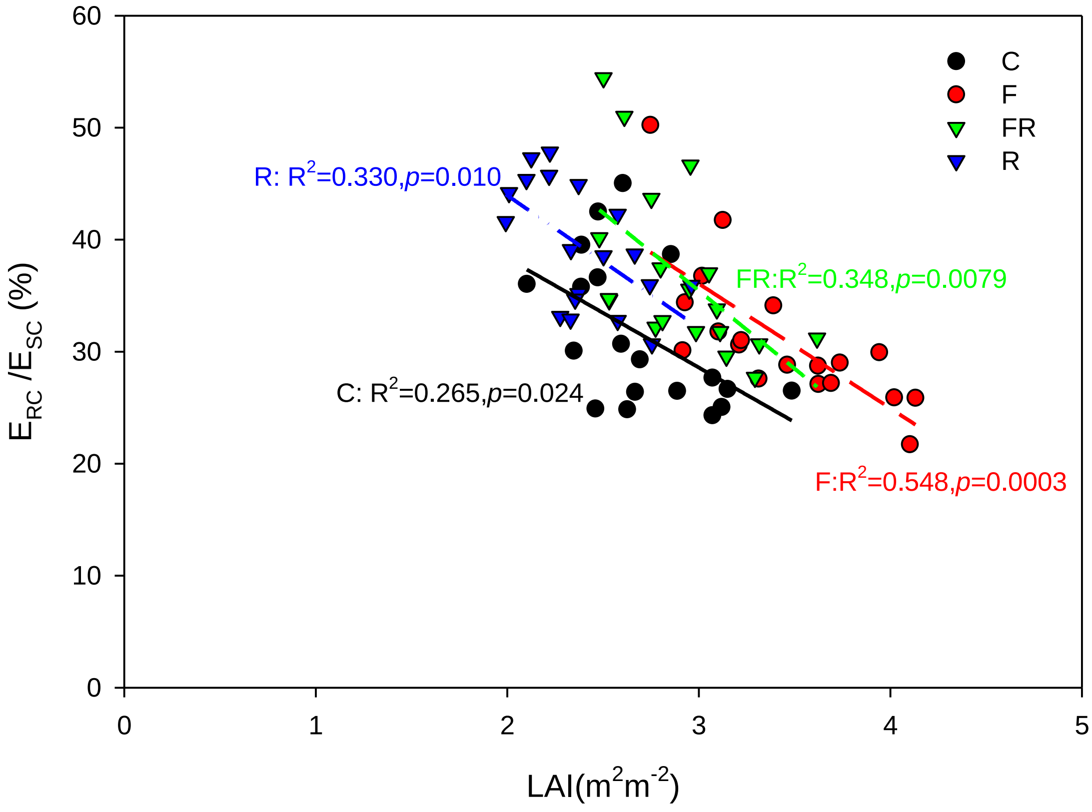

Figure 3.4. Relationships between the contribution of monthly cumulative root CO_2 efflux to the monthly cumulative soil CO_2 efflux (E_{RC} /E_{SC}) and leaf area index (*LAI*) in the four treatments. The black solid line, red dashed line, the green dashed line and blue dashed-dotted line are the regression lines between E_{RC} /E_{SC} and *LAI* in the C, F, FR, and R treatments, respectively. The abbreviations of the treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

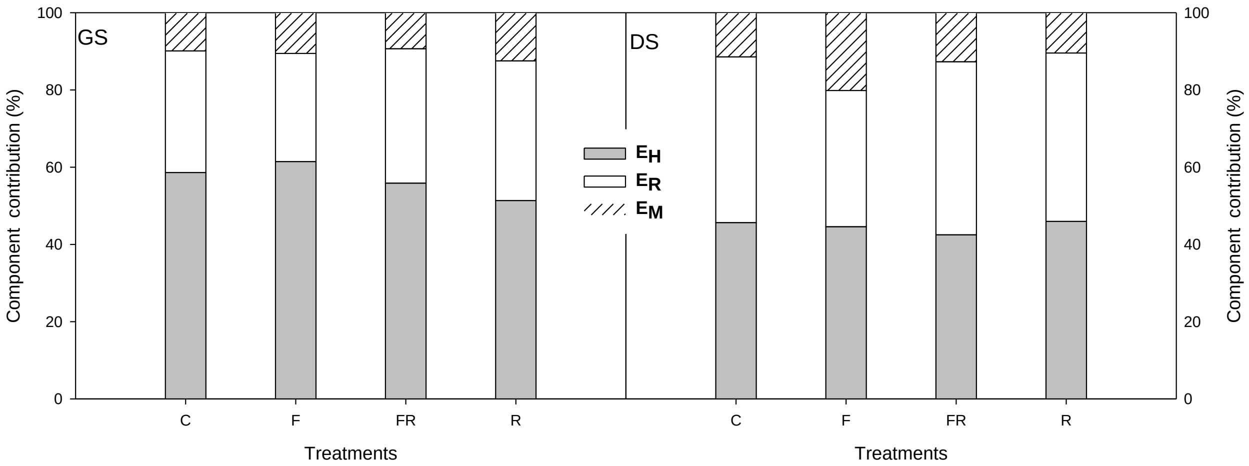

Figure 3.5. The contribution of cumulative heterotrophic CO_2 efflux (E_H, g C m^{-2}), root CO_2 efflux (E_R, g C m^{-2}) and ectomycorrhizal hyphae CO_2 efflux (E_M, g C m^{-2}) to cumulative soil CO_2 efflux (E_S, g C m^{-2}) in the growing season (GS) and the dormant season (DS). The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

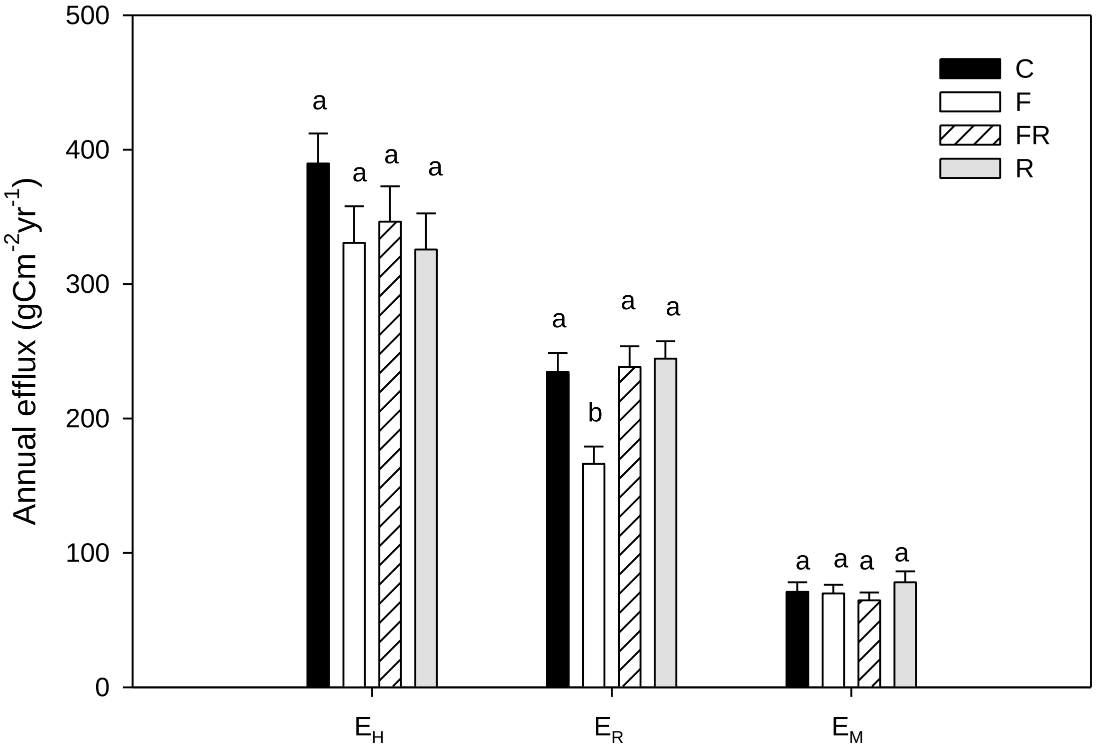

Figure 3.6. Annual heterotrophic CO_2 efflux (E_H, g C m^{-2} yr^{-1}), root CO_2 efflux (E_R, g C m^{-2} yr^{-1}), and ectomycorrhizal hyphae CO_2 efflux (E_M, g C m^{-2} yr^{-1}) in the four treatments. The abbreviations of the treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only. The different lowercase letters indicate there was a significant difference ($p<0.05$).

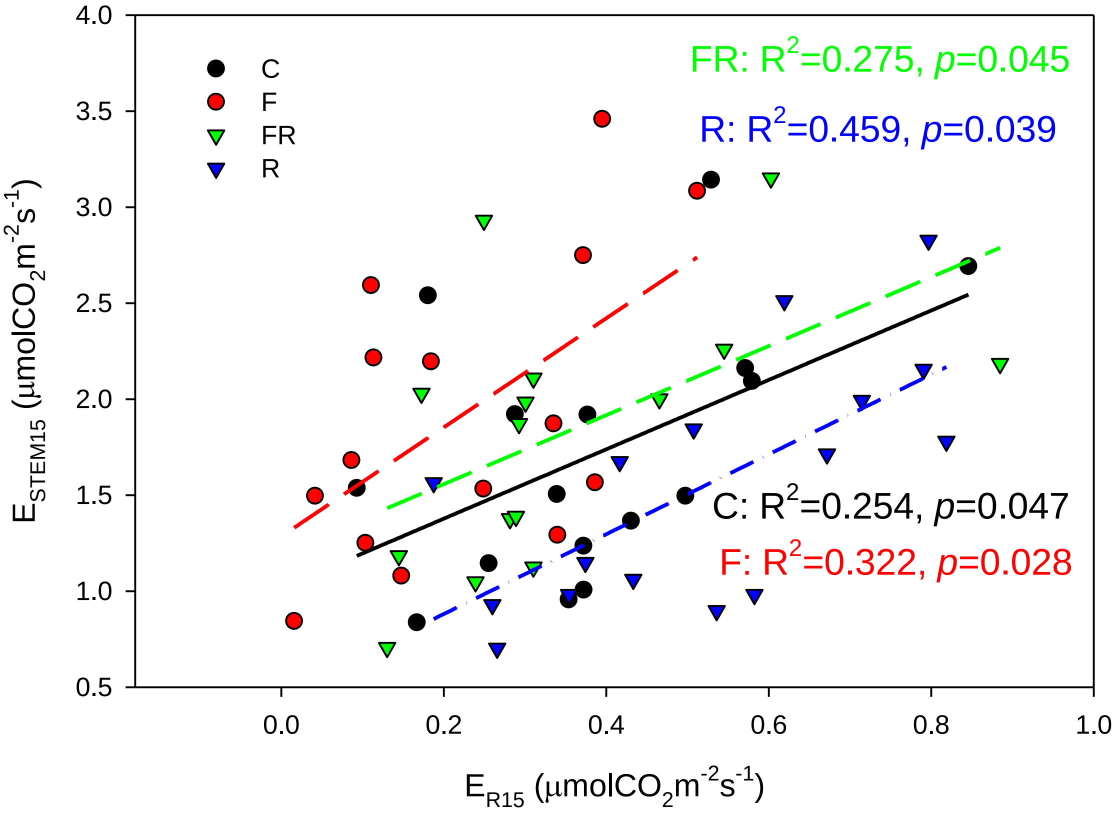

Figure 3.7. Relationships between stem CO_2 efflux normalized at 15°C (E_{STEM15}) and root CO_2 efflux normalized at 15°C (E_{R15}) in the four treatments. The black solid line, red dashed line, the green dashed line and blue dashed-dotted line are the regression lines between E_{STEM15} and E_{R15} in the C, F, FR, and R treatments, respectively. The abbreviations of the treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

Table S3.1. Relationships between stem CO_2 efflux normalized at 15°C (E_{STEM15}) and root CO_2 efflux normalized at 15°C (E_{R15}) in four treatments. The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only. The letter n indicates sample size, Adj R^2 indicates adjusted coefficient of determination.

Treatments	Equations	n	Adj R^2	*p*
C	$E_{STEM15} = 1.016 + 1.807\ E_{R15}$	16	0.253	0.047
F	$E_{STEM15} = 1.285 + 2.839\ E_{R15}$	15	0.322	0.027
FR	$E_{STEM15} = 1.198 + 1.798\ E_{R15}$	15	0.275	0.045
R	$E_{STEM15} = 0.465 + 2.079\ E_{R15}$	16	0.459	0.039

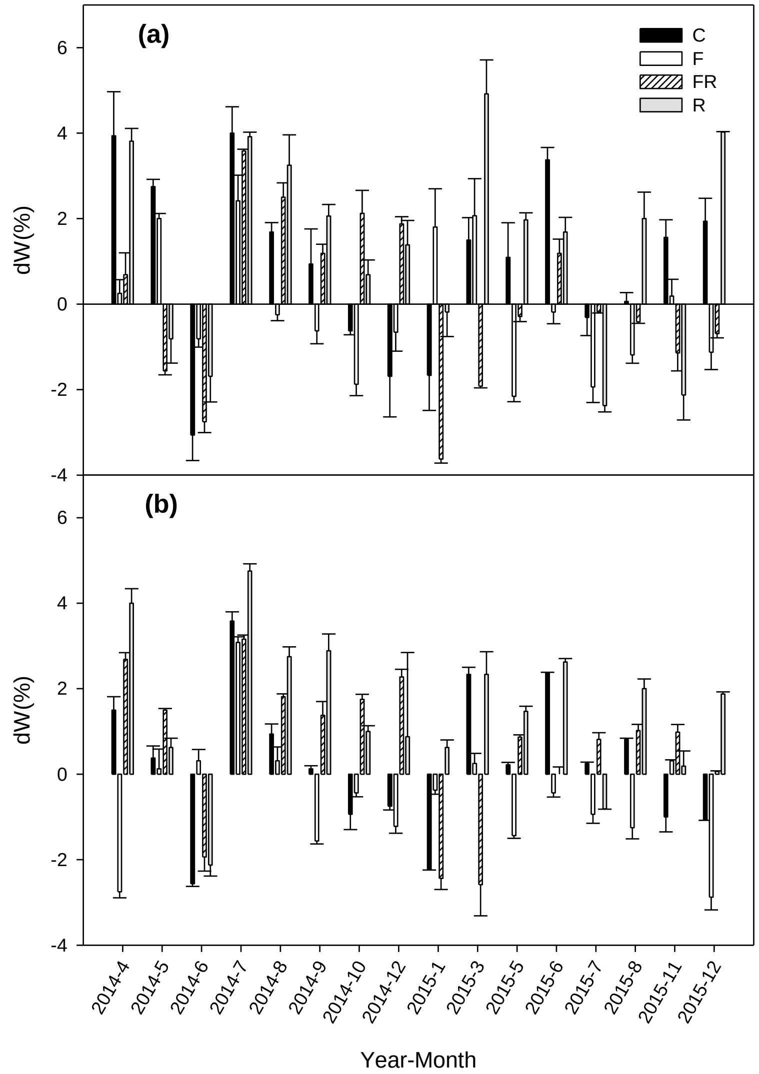

Figure S 3.1. a) Seasonal patterns of the difference in volumetric water content (*dW*) between trenched E_H subplots and untrenched E_S and b) *dW* between trenched E_{H+M} subplots and E_S plots during the total measurement period. The positive values indicate the volumetric water content in E_H/E_M subplots was higher than those in E_S plots. The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

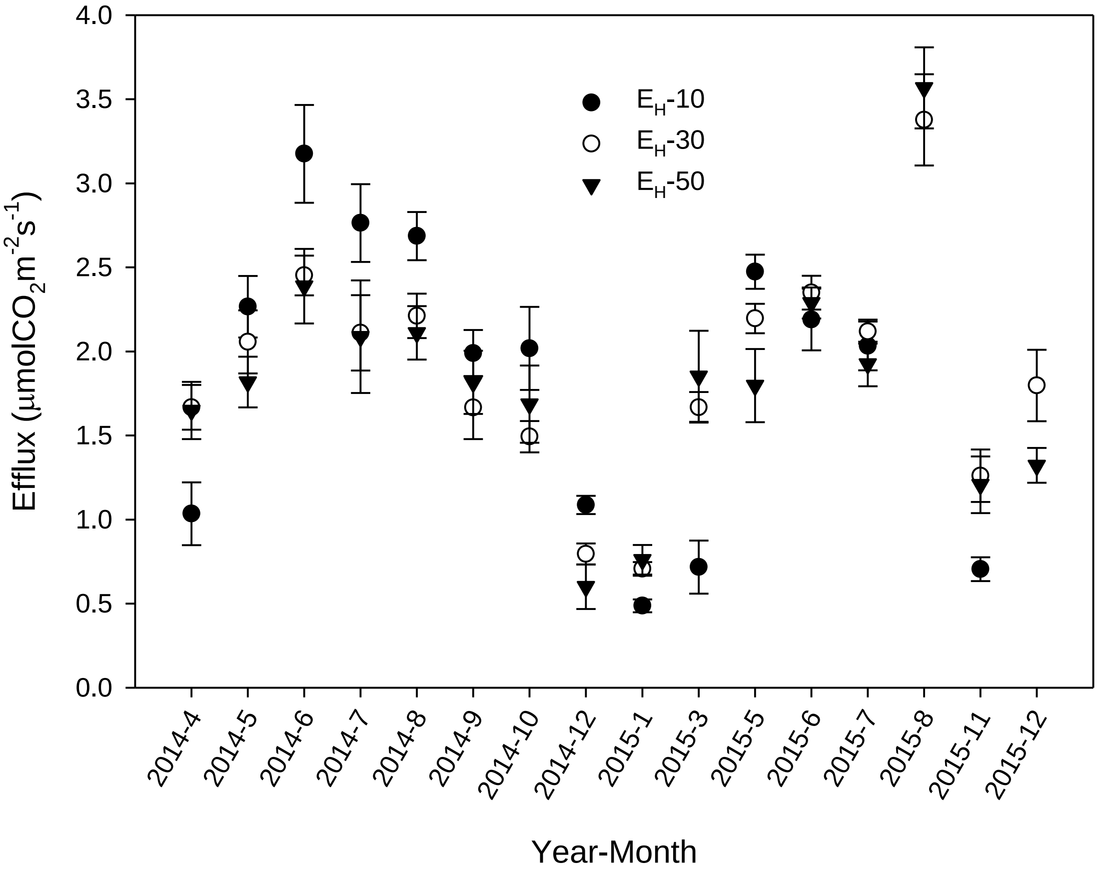

Figure S 3.2. Seasonal patterns of heterotrophic CO_2 efflux (E_H) from trenched plots of different sizes. Symbols are: E_H-10, E_H measured in steel pipes (diameter =10cm): E_H-30, measured in a trenched subplot with a 30cm ×30cm area; and E_H-50, E_H measured in a trenched subplot with a 50cm ×50cm area. Error bars represent ± 1 SEM.

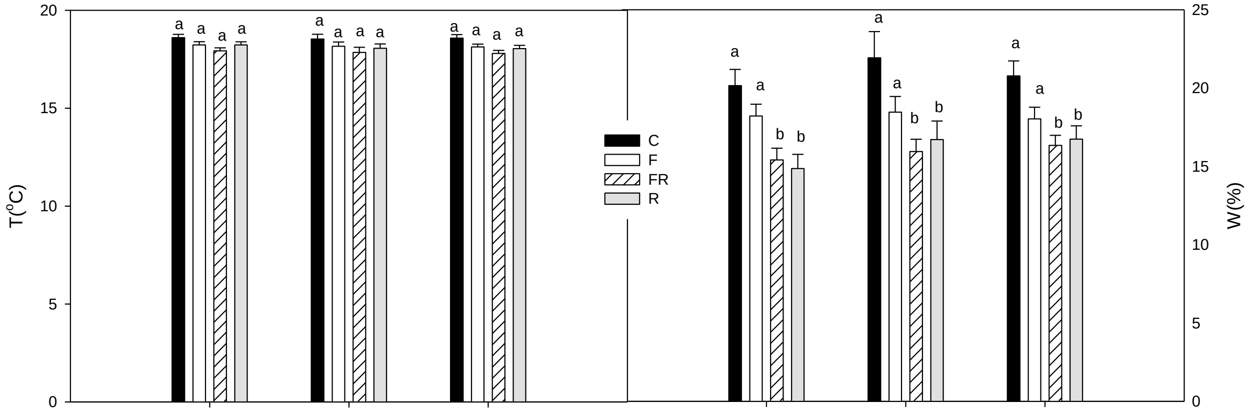

Figure S3.3. Overall means of soil temperature at 10cm soil depth (*T*) and soil volumetric water content at 20cm depth (*W*) during the total measurement period. The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only. The different lowercase letters indicate there was a statistically significant difference ($p<0.05$).

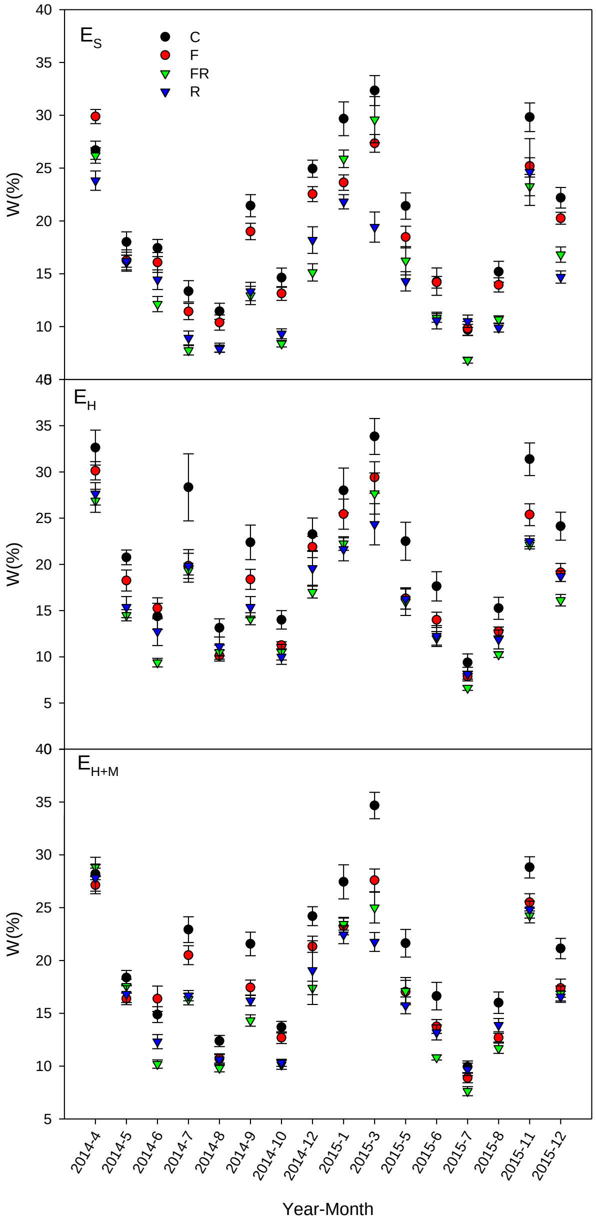

Figure S3.4. Seasonal patterns of soil volumetric water content at 20cm depth (W) in untrenched E_S plots and trenched E_H and E_{H+M} subplots in the four treatments during the total measurement period. The abbreviations of four treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only. The different lowercase letters indicate there was a statistically significant difference ($p<0.05$).

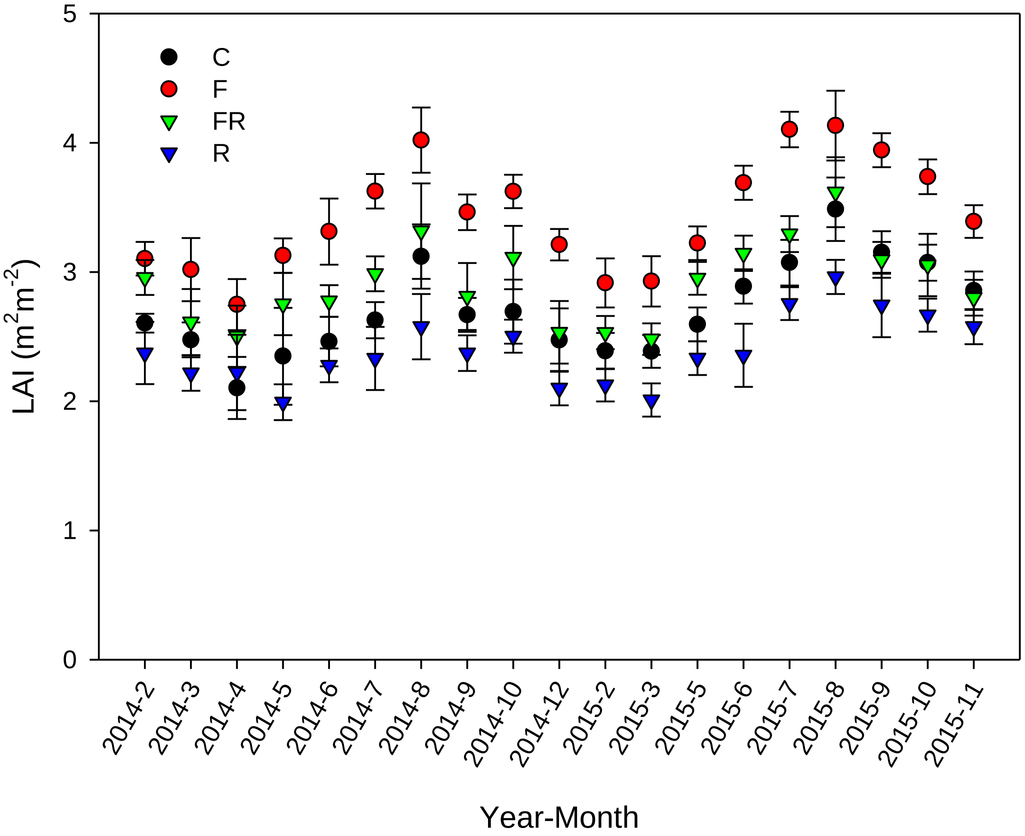

Figure S 3.5. Seasonal patterns of leaf area index (*LAI*) in the four treatments from 2014 to 2015. The abbreviations of the treatments are C, Control; F, fertilization only, FR, both fertilization and throughfall reduction; and R, throughfall reduction only.

References

Albaugh, T.J., Allen, H.L. & Fox, T.R. (2008) Nutrient use and uptake in *Pinus taeda*. *Tree physiology*, **28**, 1083-1098.

Andrew, C. & Lilleskov, E.A. (2014) Elevated CO_2 and O_3 effects on ectomycorrhizal fungal root tip communities in consideration of a post-agricultural soil nutrient gradient legacy. *Mycorrhiza*, **24**, 581-593.

Angert, A., Muhr, J., Negron Juarez, R., Alegria Muñoz, W., Kraemer, G., Ramirez Santillan, J., Barkan, E., Mazeh, S., Chambers, J.Q. & Trumbore, S.E. (2012) Internal respiration of Amazon tree stems greatly exceeds external CO_2 efflux. *Biogeosciences*, **9**, 4979-4991.

Aubrey, D.P. & Teskey, R.O. (2009) Root-derived CO_2 efflux via xylem stream rivals soil CO_2 efflux. *New Phytologist*, **184**, 35-40.

Bahn, M., Rodeghiero, M., Anderson-Dunn, M., Dore, S., Gimeno, C., Drosler, M., Williams, M., Ammann, C., Berninger, F., Flechard, C., Jones, S., Balzarolo, M., Kumar, S., Newesely, C., Priwitzer, T., Raschi, A., Siegwolf, R., Susiluoto, S., Tenhunen, J., Wohlfahrt, G. & Cernusca, A. (2008) Soil Respiration in European Grasslands in Relation to Climate and Assimilate Supply. *Ecosystems*, **11**, 1352-1367.

Bartkowiak, S.M., Samuelson, L.J., McGuire, M.A. & Teskey, R.O. (2015) Fertilization increases sensitivity of canopy stomatal conductance and transpiration to throughfall reduction in an 8-year-old loblolly pine plantation. *Forest Ecology and Management*, **354**, 87-96.

Bloemen, J., McGuire, M.A., Aubrey, D.P., Teskey, R.O. & Steppe, K. (2013) Transport of root-respired CO_2 via the transpiration stream affects aboveground carbon assimilation and CO_2 efflux in trees. *New Phytologist*, **197**, 555-65.

Bond-Lamberty, B., Wang, C. & Gower, S.T. (2004) A global relationship between the heterotrophic and autotrophic components of soil respiration? *Global Change Biology*, **10**, 1756-1766.

Borken, W., Savage, K., Davidson, E.A. & Trumbore, S.E. (2006) Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biology*, **12**, 177-193.

Burton, A.J., Jarvey, J.C., Jarvi, M.P., Zak, D.R. & Pregitzer, K.S. (2012) Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. *Global Change Biology*, **18**, 258-266.

Chen, G., Yang, Y. & Robinson, D. (2014) Allometric constraints on, and trade-offs in, belowground carbon allocation and their control of soil respiration across global forest ecosystems. *Global Change Biology*, **20**, 1674-1684.

Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, R., Jones, R., Kolli, R.K., Kwon, W. & Laprise, R. (2007) Regional climate projections. Climate Change, 2007: The Physical Science Basis. Contribution of Working group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, University Press, Cambridge, Chapter 11, 847-940.

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A. & Lindahl, B.D. (2015) Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist*, **205**, 1525-1536.

Cudlín, P., Kieliszewska-Rokicka, B., Rudawska, M., Grebenc, T., Alberton, O., Lehto, T., Bakker, M.R., Børja, I., Konôpka, B. & Leski, T. (2007) Fine roots and ectomycorrhizas as indicators of environmental change. *Plant Biosystems*, **141**, 406-425.

Curiel yuste, J., Janssens, I.A., Carrara, A. & Ceulemans, R. (2004) Annual Q_{10} of soil respiration reflects plant phenological patterns as well as temperature sensitivity. *Global Change Biology*, **10**, 161-169.

de Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M. & Bardgett, R.D. (2012) Land use alters the resistance and resilience of soil food webs to drought. *Nature Climate Change*, **2**, 276-280.

Domec, J.C., King, J.S., Noormets, A., Treasure, E., Gavazzi, M.J., Sun, G. & McNulty, S.G. (2010) Hydraulic redistribution of soil water by roots affects whole-stand evapotranspiration and net ecosystem carbon exchange. *New Phytologist*, **187**, 171-183.

Domec, J.C., Ogee, J., Noormets, A., Jouangy, J., Gavazzi, M., Treasure, E., Sun, G., McNulty, S.G. & King, J.S. (2012) Interactive effects of nocturnal transpiration and climate change on the root hydraulic redistribution and carbon and water budgets of southern United States pine plantations. *Tree Physiology*, **32**, 707-23.

Drake, J.E., Stoy, P.C., Jackson, R.B. & DeLucia, E.H. (2008) Fine-root respiration in a loblolly pine (*Pinus taeda* L.) forest exposed to elevated CO_2 and N fertilization. *Plant Cell Environment*, **31**, 1663-72.

Edwards, I.P., Zak, D.R., Kellner, H., Eisenlord, S.D. & Pregitzer, K.S. (2011) Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. *PloS one*, **6**, e20421.

Fahey, T.J., Tierney, G.L., Fitzhugh, R.D., Wilson, G.F. & Siccama, T.G. (2005) Soil respiration and soil carbon balance in a northern hardwood forest ecosystem. *Canadian Journal of Forest Research*, **35**, 244-253.

Fox, T.R., Allen, H.L., Albaugh, T.J., Rubilar, R. & Carlson, C.A. (2007) Tree nutrition and forest fertilization of pine plantations in the southern United States. *Southern Journal of Applied Forestry*, **31**, 5-11.

Gallo, M., Amonette, R., Lauber, C., Sinsabaugh, R. & Zak, D. (2004) Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microbial Ecology*, **48**, 218-229.

George, K., Norby, R.J., Hamilton, J.G. & DeLucia, E.H. (2003) Fine-root respiration in a loblolly pine and sweetgum forest growing in elevated CO_2. *New Phytologist*, **160**, 511-522.

Gonzalez-Benecke, C.A., Gezan, S.A., Albaugh, T.J., Allen, H.L., Burkhart, H.E., Fox, T.R., Jokela, E.J., Maier, C.A., Martin, T.A. & Rubilar, R.A. (2014) Local and general above-stump biomass functions for loblolly pine and slash pine trees. *Forest Ecology and Management*, **334**, 254-276.

Gough, C.M. & Seiler, J.R. (2004) The influence of environmental, soil carbon, root, and stand characteristics on soil CO_2 efflux in loblolly pine (*Pinus taeda* L.) plantations located on the South Carolina Coastal Plain. *Forest Ecology and Management*, **191**, 353-363.

Hanson, P., Edwards, N., Garten, C. & Andrews, J. (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*, **48**, 115-146.

Hasselquist, N.J., Metcalfe, D.B. & Högberg, P. (2012) Contrasting effects of low and high nitrogen additions on soil CO_2 flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. *Global Change Biology*, **18**, 3596-3605.

Haynes, B.E. & Gower, S.T. (1995) Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiology*, **15**, 317-325.

Heinemeyer, A. & McNamara, N.P. (2011) Comparing the closed static versus the closed dynamic chamber flux methodology: Implications for soil respiration studies. *Plant and Soil*, **346**, 145-151.

Heinemeyer, A., Hartley, I.P., Evans, S.P., Carreira De La Fuente, J.A. & Ineson, P. (2007) Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology*, **13**, 1786-1797.

Heinemeyer, A., Wilkinson, M., Vargas, R., Subke, J.A., Casella, E., Morison, J.I.L. and Ineson, P., 2012. Exploring the “overflow tap” theory: linking forest soil CO_2 fluxes and individual mycorrhizosphere components to photosynthesis. *Biogeosciences*, **8**, 79-95.

Hendricks, J.J., Mitchell, R.J., Kuehn, K.A. & Pecot, S.D. (2016) Ectomycorrhizal fungal mycelia turnover in a longleaf pine forest. *New Phytologist*, **209**:1693-1704.

Hibbard, K.A., Law, B.E., Reichstein, M. & Sulzman, J. (2005) An analysis of soil respiration across northern hemisphere temperate ecosystems. *Biogeochemistry,* **73**, 29-70.

Hinko-Najera, N., Fest, B., Livesley, S.J. & Arndt, S.K. (2015) Reduced throughfall decreases autotrophic respiration, but not heterotrophic respiration in a dry temperate broadleaved evergreen forest. *Agricultural and Forest Meteorology*, **200**, 66-77.

Hopkins, F., Gonzalez-Meler, M.A., Flower, C.E., Lynch, D.J., Czimczik, C., Tang, J. & Subke, J.-A. (2013) Ecosystem-level controls on root-rhizosphere respiration. *New Phytologist*, **199**, 339-351.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J.

& Law, B.E. (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, **3**, 315-322.

King, J.S., Albaugh, T.J., Allen, H.L., Buford, M., Strain, B.R. & Dougherty, P. (2002) Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. *New Phytologist*, **154**, 389-398.

Kunkel, K.E. & Frankson, R.M. (2015) Global Land Surface Extremes of Precipitation: Data Limitations and Trends. *Journal of Extreme Events*, 1550004.

Langley A, J., Chapman, S.K. & Hungate, B.A. (2006) Ectomycorrhizal colonization slows root decomposition: the post-mortem fungal legacy. *Ecology Letters*, **9**, 955-959.

Lichter, J., Billings, S.A., Ziegler, S.E., Gaindh, D., Ryals, R., Finzi, A.C., Jackson, R.B., Stemmler, E.A. & Schlesinger, W.H. (2008) Soil carbon sequestration in a pine forest after 9 years of atmospheric CO_2 enrichment. *Global Change Biology*, **14**, 2910-2922.

Lindroth, A., Lagergren, F., Aurela, M., Bjarnadottir, B., Christensen, T., Dellwik, E., Grelle, A., Ibrom, A., Johansson, T., Lankreijer, H., Launiainen, S., Laurila, T., MÖLder, M., Nikinmaa, E., Pilegaard, K.I.M., Sigurdsson, B.D. & Vesala, T. (2008) Leaf area index is the principal scaling parameter for both gross photosynthesis and ecosystem respiration of Northern deciduous and coniferous forests. *Tellus B*, **60**, 129-142.

Litton, C.M., Raich, J.W. & Ryan, M.G. (2007) Carbon allocation in forest ecosystems. *Global Change Biology*, **13**, 2089-2109.

Liu, L. & Greaver, T.L. (2010) A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters*, **13**, 819-28.

Liu, L., Wang, X., Lajeunesse, M.J., Miao, G., Piao, S., Wan, S., Wu, Y., Wang, Z., Yang, S., Li, P. & Deng, M. (2016) A cross-biome synthesis of soil respiration and its determinants under simulated precipitation changes. *Global Change Biology*, **22**, 1394-405.

Lu, M., Zhou, X., Luo, Y., Yang, Y., Fang, C., Chen, J. & Li, B. (2011) Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. *Agriculture, Ecosystems & Environment*, **140**, 234-244.

Maier, C.A. & Kress, L.W. (2000) Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability. *Canadian Journal of Forest Research*, **30**, 347-359.

Maier, C.A. & Clinton, B.D. (2006) Relationship between stem CO_2 efflux, stem sap velocity and xylem CO_2 concentration in young loblolly pine trees. *Plant Cell and Environment*, **29**, 1471-1483.

Maier, C.A., Albaugh, T.J., Lee Allen, H. & Dougherty, P.M. (2004) Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: the effect of site resources on the stand carbon balance. *Global Change Biology*, **10**, 1335-1350.

Moore, D.J., Gonzalez-Meler, M.A., Taneva, L., Pippen, J.S., Kim, H.S. & Delucia, E.H. (2008) The effect of carbon dioxide enrichment on apparent stem respiration from *Pinus taeda* L. is confounded by high levels of soil carbon dioxide. *Oecologia*, **158**, 1-10.

Neumann, J. & Matzner, E. (2014) Contribution of newly grown extramatrical ectomycorrhizal mycelium and fine roots to soil respiration in a young Norway spruce site. *Plant and Soil*, **378**, 73-82.

Ngao, J., Epron, D., Delpierre, N., Bréda, N., Granier, A. & Longdoz, B. (2012) Spatial variability of soil CO_2 efflux linked to soil parameters and ecosystem characteristics in a temperate beech forest. *Agricultural and Forest Meteorology*, **154-155**, 136-146.

Nilsson, L.O. & Wallander, H. (2003) Production of external mycelium by ectomycorrhizal fungi in a norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist*, **158**, 409-416.

Noormets, A., Gavazzi, M.J., McNulty, S.G., Domec, J.-C., Sun, G.E., King, J.S. & Chen, J. (2010) Response of carbon fluxes to drought in a coastal plain loblolly pine forest. *Global Change Biology*, **16**, 272-287.

Oishi, A.C., Palmroth, S., Butnor, J.R., Johnsen, K.H. & Oren, R. (2013) Spatial and temporal variability of soil CO_2 efflux in three proximate temperate forest ecosystems. *Agricultural and Forest Meteorology*, **171-172**, 256-269.

Olsson, P., Linder, S., Giesler, R. & Högberg, P. (2005) Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. *Global Change Biology*, **11**, 1745-1753.

Palmroth, S., Oren, R., McCarthy, H.R., Johnsen, K.H., Finzi, A.C., Butnor, J.R., Ryan, M.G. & Schlesinger, W.H. (2006) Aboveground sink strength in forests controls the allocation of carbon below ground and its [CO_2]-induced enhancement. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 19362-7.

Phillips, R.P., Bernhardt, E.S. & Schlesinger, W.H. (2009) Elevated CO_2 increases root exudation from loblolly pine (*Pinus taeda*) seedlings as an N-mediated response. *Tree physiology*, **29**, 1513-1523.

Phillips, R.P., Meier, I.C., Bernhardt, E.S., Grandy, A.S., Wickings, K. & Finzi, A.C. (2012) Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. *Ecology Letters*, **15**, 1042-9.

Pritchard, S.G., Taylor, B.N., Cooper, E.R., Beidler, K.V., Strand, A.E., McCormack, M.L. & Zhang, S. (2014) Long-term dynamics of mycorrhizal root tips in a loblolly pine forest grown with free-air CO_2 enrichment and soil N fertilization for 6 years. *Global Change Biology*, **20**, 1313-26.

Reichstein, M., Rey, A., Freibauer, A., Tenhunen, J., Valentini, R., Banza, J., Casals, P., Cheng, Y., Grünzweig, J.M., Irvine, J., Joffre, R., Law, B.E., Loustau, D., Miglietta, F., Oechel, W., Ourcival, J.-M., Pereira, J.S., Peressotti, A., Ponti, F., Qi, Y., Rambal, S., Rayment, M., Romanya, J., Rossi, F., Tedeschi, V., Tirone, G., Xu, M. & Yakir, D. (2003) Modeling temporal and large-scale spatial variability of soil respiration from soil water availability, temperature and vegetation productivity indices. *Global Biogeochemical Cycles*, **17**.

Retzlaff, W.A., Handest, J.A., O'Malley, D.M., McKeand, S.E. & Topa, M.A. (2001) Whole-tree biomass and carbon allocation of juvenile trees of loblolly pine (*Pinus taeda*): influence of genetics and fertilization. *Canadian Journal of Forest Research*, **31**, 960-970.

Risk, D., Nickerson, N., Phillips, C.L., Kellman, L. & Moroni, M. (2012) Drought alters respired $\delta^{13}CO_2$ from autotrophic, but not heterotrophic soil respiration. *Soil Biology and Biochemistry*, **50**, 26-32.

Samuelson, L., Mathew, R., Stokes, T., Feng, Y., Aubrey, D. & Coleman, M. (2009) Soil and microbial respiration in a loblolly pine plantation in response to seven years of irrigation and fertilization. *Forest Ecology and Management*, **258**, 2431-2438.

Samuelson, L.J., Pell, C.J., Stokes, T.A., Bartkowiak, S.M., Akers, M.K., Kane, M., Markewitz, D., McGuire, M.A. & Teskey, R.O. (2014) Two-year throughfall and fertilization effects on leaf physiology and growth of loblolly pine in the Georgia Piedmont. *Forest Ecology and Management*, **330**, 29-37.

Schimel, J., Balser, T.C. & Wallenstein, M. (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology*, **88**, 1386-1394.

Schindlbacher, A., Wunderlich, S., Borken, W., Kitzler, B., Zechmeister-Boltenstern, S. & Jandl, R. (2012) Soil respiration under climate change: prolonged summer drought offsets soil warming effects. *Global Change Biology*, **18**, 2270-2279.

Scott-Denton, L.E., Rosenstiel, T.N. & Monson, R.K. (2006) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biology*, **12**, 205-216.

Seager, R., Tzanova, A. & Nakamura, J. (2009) Drought in the Southeastern United States: Causes, Variability over the Last Millennium, and the Potential for Future Hydroclimate Change. *Journal of Climate*, **22**, 5021-5045.

Shi, Z., Thomey, M.L., Mowll, W., Litvak, M., Brunsell, N.A., Collins, S.L., Pockman, W.T., Smith, M.D., Knapp, A.K. & Luo, Y. (2014) Differential effects of extreme drought on production and respiration: synthesis and modeling analysis. *Biogeosciences*, **11**, 621-633.

Subke, J.A., Inglima, I. & Francesca Cotrufo, M. (2006) Trends and methodological impacts in soil CO_2 efflux partitioning: A meta-analytical review. *Global Change Biology*, **12**, 921-943.

Subke, J.-A., Voke, N.R., Leronni, V., Garnett, M.H. & Ineson, P. (2011) Dynamics and pathways of autotrophic and heterotrophic soil CO_2 efflux revealed by forest girdling. *Journal of Ecology*, **99**, 186-193.

Suseela, V., Conant, R.T., Wallenstein, M.D. & Dukes, J.S. (2012) Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Global Change Biology*, **18**, 336-348.

Taylor, B.N., Strand, A.E., Cooper, E.R., Beidler, K.V., Schonholz, M. & Pritchard, S.G. (2014) Root length, biomass, tissue chemistry and mycorrhizal colonization following 14 years of CO_2 enrichment and 6 years of N fertilization in a warm temperate forest. *Tree Physiology*, **34**, 955-65.

Templeton, B.S., Seiler, J.R., Peterson, J.A. & Tyree, M.C. (2015) Environmental and stand management influences on soil CO_2 efflux across the range of loblolly pine. *Forest Ecology and Management*, **355**, 15-23.

Teste, F.P., Lieffers, V.J. & Strelkov, S.E. (2012) Ectomycorrhizal community responses to intensive forest management: thinning alters impacts of fertilization. *Plant and Soil*, **360**, 333-347.

Treseder, K.K. (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters*, **11**, 1111-20.

Templeton, B.S. (2009) Environmental And Stand Variables Influencing Soil CO_2 Efflux Across The Managed Range Of Loblolly Pine. Master Thesis.

Tyree, M.C., Seiler, J.R., Aust, W.M., Sampson, D.A. & Fox, T.R. (2006) Long-term effects of site preparation and fertilization on total soil CO_2 efflux and heterotrophic respiration in a

33-year-old *Pinus taeda* L. plantation on the wet flats of the Virginia Lower Coastal Plain. *Forest Ecology and Management*, **234**, 363-369.

Ubierna, N., Kumar, A.S., Cernusak, L.A., Pangle, R.E., Gag, P.J. & Marshall, J.D. (2009) Storage and transpiration have negligible effects on ^{13}C of stem CO_2 efflux in large conifer trees. *Tree Physiology*, **29**, 1563-1574.

Vallack, H.W., Leronni, V., Metcalfe, D.B., Högberg, P., Ineson, P. & Subke, J.-A. (2012) Application of nitrogen fertilizer to a boreal pine forest has a negative impact on the respiration of ectomycorrhizal hyphae. *Plant and Soil*, **352**, 405-417.

Wallander, H., Ekblad, A. & Bergh, J. (2011) Growth and carbon sequestration by ectomycorrhizal fungi in intensively fertilized Norway spruce forests. *Forest Ecology and Management*, **262**, 999-1007.

Williams, M.A. (2007) Response of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils. *Soil Biology and Biochemistry*, **39**, 2750-2757.

Xu, M., DeBiase, T.A. & Qi, Y. (2000) A simple technique to measure stem respiration using a horizontally oriented soil chamber. *Canadian Journal of Forest Research*, **30**, 1555-1560.

Yang, J., He, Y., Aubrey, D.P., Zhuang, Q. & Teskey, R.O. (2016) Global patterns and predictors of stem CO_2 efflux in forest ecosystems. *Global Change Biology*, **22**, 1433-1444.

Yang, J.Y., Teskey, R.O. & Wang, C.K. (2012) Stem CO_2 efflux of ten species in temperate forests in Northeastern China. *Trees*, **26**, 1225-1235.

Yuste, J.C., PeÑUelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M. & Sardans, J. (2011) Drought-resistant fungi control soil organic matter decomposition and its response to temperature. *Global Change Biology*, **17**, 1475-1486.

Zak, D.R., Pregitzer, K.S., Burton, A.J., Edwards, I.P. & Kellner, H. (2011) Microbial responses to a changing environment: implications for the future functioning of terrestrial ecosystems. *Fungal Ecology*, **4**, 386-395.

Zhou, L., Zhou, X., Zhang, B., Lu, M., Luo, Y., Liu, L. & Li, B. (2014) Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. *Global Chang Biology*, **20**, 2332-43.

CHAPTER 4

CONCLUSIONS

Summary of Previous Chapters

The objectives of this research were to quantify the responses soil CO_2 efflux (E_S) and its components and total belowground carbon flux (*TBCF*) to increasing soil fertility and decreasing soil water moisture. The experiments were carried out in a 7-year-old loblolly pine (*Pinus taeda* L.) plantation. The first experiment examined the effects of fertilization (control and fertilization) and throughfall reduction (control and 30% throughfall reduction) on soil CO_2 efflux and *TBCF*. The second experiment determined the sensitivity of the components of soil CO_2 efflux (heterotrophic CO_2 efflux, E_H; root CO_2 efflux, E_R; and CO_2 efflux from ectomycorrhizal hyphae, E_M) to fertilization and throughfall reduction, and to quantify relationships between soil temperature, moisture and other factors on E_S, E_H, E_R and E_M. Besides CO_2 efflux measurements, litterfall, leaf area index and aboveground and belowground production were also measured in both studies.

In the first experiment, fertilization reduced the grand mean of E_S in the total measurement period, mean E_S in the growing season. Throughfall reduction and the interaction between fertilization and throughfall reduction had no effect on E_S in the four treatments, although throughfall reduction significantly decreased soil moisture by 20%. The moderate, but not severe, drought caused by the 30% throughfall reduction, and high interannual variation in precipitation, contributed to the small effect of throughfall reduction on E_S and its components. However, soil moisture did affect E_S in our study. The close correlations between E_S normalized to 15°C (E_{15}) and soil moisture were observed in all treatments except throughfall reduction. Soil temperature, soil moisture and their interaction accounted for 51% to 74% of the variation in E_S in all treatments. In addition, the apparent Q_{10} of E_S was higher in the non-drought period than in the drought period. Cumulative E_S in the dormant season was between 16% and 19% of annual cumulative E_S. The average annual cumulative E_S in the treatments was 544 g C m^{-2} yr^{-1} (fertilization), 645 g C m^{-2} yr^{-1} (fertilization and throughfall reduction), 665 g C m^{-2} yr^{-1} (throughfall reduction) and 695 g C m^{-2} yr^{-1} (control). Compared to the control treatment, fertilization significantly reduced annual cumulative E_S by 21%. The reduction in E_S was related with a decrease in E_A. However, the definitive cause for the decrease in E_A could not be identified because of a lack of direct measurements of fine root biomass, fine root lifespan, and ectomycorrhizal fungi production.

The average annual *TBCF* was 532 g C m^{-2} yr^{-1} for fertilization, 634 g C m^{-2} yr^{-1} for fertilization and throughfall reduction, 662 g C m^{-2} yr^{-1} for throughfall reduction and 685 g C m^{-2} yr^{-1} for control treatment. Fertilization also had significant effects on annual E_S, litterfall, and *TBCF*. Compared to the control, fertilization decreased *TBCF* by 22%, caused by decreased annual E_S and increased litterfall. Stem and branch production were linearly negatively related to *TBCF*. The response of *TBCF* to fertilization was likely due to a shift of carbon allocation which favored aboveground over belowground carbon allocation (Giardina et al., 2002; Maier et al., 2004; Litton et al., 2007; Chen et al., 2014).

In the second experiment, both E_S and E_R were significantly reduced by fertilization while throughfall reduction had no effect on E_H, E_R and E_M. A shift in production from fine roots to coarse roots and the long lifespan of mycorrhizal fine roots may have led to the reduction in

E_R. However, a lack of response to the throughfall reduction treatment did not mean that soil moisture had no effect on E_S, E_A or E_H. After controlling soil temperature, E_S normalized to 15^{o}C (E_{S15}) and E_H normalized at 15 ^{o}C (E_{H15}) increased with increasing soil moisture in C and F treatments. Soil temperature also explained more variation in E_S, E_H and E_R the in non-drought periods compared to the drought periods were predicted well by Soil temperature, moisture and their interaction explained from 45% to 64% of the variability in E_S and E_H in the total measurement period. However, across the same time period these factors explained < 35% of the variation in E_R and < 12% in E_M. The correlation between E_H and *LAI* suggested that there was a relationship between the *GPP* and E_S. The contribution of E_H to E_S was from 50 to 58% and the contribution of E_M to E_S was from 10% to 12% in all treatments.

We concluded that E_M was an important component of $E_{S.}$ The quantification of E_M improves our understanding and estimation of E_R in temperate forests dominated by ectomycorrhizae. We also urge caution when interpreting the small effect of throughfall reduction on E_S and its components in this study with respect to how they may respond to future climate regimes due to the presence of several interacting factors (e.g. soil temperature, *LAI*). These factors complicate predictions of the effect of changes in soil moisture on E_S and its components.

References

Chen, G., Yang, Y. & Robinson, D. (2014) Allometric constraints on, and trade-offs in, belowground carbon allocation and their control of soil respiration across global forest ecosystems. *Global Change Biology*, **20**, 1674-1684.

Giardina, C.P. & Ryan, M.G. (2002) Total Belowground Carbon Allocation in a Fast-growing Eucalyptus Plantation Estimated Using a Carbon Balance Approach. *Ecosystems*, **5**, 487-499.

Litton, C.M., Raich, J.W. & Ryan, M.G. (2007) Carbon allocation in forest ecosystems. *Global Change Biology*, **13**, 2089-2109.

Maier, C.A., Albaugh, T.J., Lee Allen, H. & Dougherty, P.M. (2004) Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: the effect of site resources on the stand carbon balance. *Global Change Biology*, **10**, 1335-1350.