TREE-BASED INTERCROPPING SYSTEMS: A POTENTIAL LAND-USE SOLUTION FOR CLIMATE CHANGE MITIGATION IN CANADIAN AGRICULTURAL LANDSCAPES

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Introduction

In tree-based intercropping (TBI) systems, the potential influence of trees in relation to carbon (C) sequestration and Greenhouse Gas (GHG) emissions reduction has been documented but the mechanisms remain poorly understood, especially for below-ground processes. Recently, several studies in this area were undertaken in Ontario, Canada under the auspices of Canada's involvement in the Global Research Alliance. C sequestration potential, nitrous oxide reduction potential and soil voids were quantified in a 25-year-old TBI system in southern Ontario for five tree species: hybrid poplar (*Populus* spp.), Norway spruce (*Picea abies*), red oak (*Quercus rubra*), black walnut (*Juglans nigra*), and white cedar (*Thuja occidentalis*), which were intercropped with soybean (*Glycine max*). Results were compared with an adjacent conventional agricultural system in which soybean was grown as the sole crop.

Materials and methods

This study was conducted in 2012 and 2013 at the University of Guelph Agroforestry Research Station: 30 ha of agricultural land established in 1987 in southwestern Ontario (43°32'28" N, 80°12' 32" W; **Figure 1**). Mean annual temperature is 7.2°C with 136 mean annual frost-free days and mean precipitation of 833 mm (344 mm of which fall during the growing season). The site has a Canadian Land Classification Index of 3 and the soil is an Albic Luvisol with a sandy loam texture (65% sand, 25% silt, 10% clay) and pH of 7.4. The fields are tilled annually to 15 cm in the spring and are tile-drained. Both systems have the same crop rotation of corn (*Zea mays*), soybean (*Glycine max*) and wheat (*Triticum vulgare*) and fertilizer rates are applied based on Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) recommendations. To determine the above- and belowground C content of five tree species, three trees of each species (3 trees x 5 species = 15 trees in total) were destructively harvested by chainsaw. Belowground roots were excavated to a depth of 1.5 m using a 580 Robert Tire Backhoe. All roots, having a diameter greater than 5 mm, from a soil volume of 24 m^3 [4 m along the tree row (2 m north and 2 m south from the tree trunk) and 4 m into the crop alleys (2 m east and 2 m west from the tree trunk) and a depth of 1.5m], were hand-picked. Distances from the tree trunk were determined from a previous study by Gray (2000), who determined that 90% of the roots on this site were within the above given distances from the trunk and also in the indicated soil volume.

Figure 1. University of Guelph, Agroforestry Research Station aerial view, Guelph, Ontario, Canada.

DNA was extracted from soil cores collected around four of the tree species (walnut, red oak, Norway spruce, poplar) and used for quantitative real-time PCR to determine the abundance of key functional genes in the nitrification and denitrification pathways. Soil samples were collected from the tree row of the respective tree species, and up to 6 m into the cropping alley at different distances from the tree row.
To characterize the soil surface (top 3.5 cm) microstructure, soils adjacent to walnut, poplar, red oak, Norway spruce and soybeans were analysed using X-ray computer microtomography. This

was used to evaluate soil void phase characteristics and heterogeneity of soil matrix. The ImageJ plug-in, Analyze Particles (Doube et al. 2010), was used, and followed by calculations within Microsoft Excel (2010) to determine void sizes. Voids were segregated into large inter-macroaggregate (> 150,000 voxels or 32.4 mm^3), medium intra-aggregate (8-150,000 voxels or 0.002-32.4 mm^3), and small intra-aggregate voids (< 8 voxels or < 0.002 mm^3) to facilitate processing.

Results and discussion

The net C flux for poplar, spruce, oak, walnut, cedar and the soybean sole-crop were + 2.1, + 1.6, + 0.8, + 1.8, +1.4 and − 1.2 t C ha^{-1}, y^{-1}, respectively (**Table 1**). The results suggest a greater atmospheric CO_2 sequestration potential for all five tree species when compared to a conventional agricultural system. The quantification of C inputs and outputs helps to determine which species may be best suited for TBI systems in southern Ontario, Canada. Previous studies have shown that fast-growing hybrid poplar trees are able to sequester more than twice as much C than a slow-growing species, such as Norway spruce (Peichl et al. 2006) in both C stocks and annual assimilation rate. However, at 25 years after establishment, hybrid poplar has reached its maturity and has started to experience branch dieback. This may explain the slight increase in the total hybrid poplar C pools from 96.5 (Peichl et al., 2006) to only 113.4 t C ha^{-1} between 13 (year 2000) and 25 (year 2012) years after establishment, respectively. However, slower growing species such as Norway spruce are continuing to add significantly higher amounts of C from 75.3 t C ha^{-1} at 13 years (Peichl et al., 2006) to 91.3 t C ha^{-1} at 25 years after establishment. Therefore, Norway spruce, and other slower growing species with longer life spans will continue to sequester atmospheric CO_2 until harvest (60 years or more). In order to calculate the total C sequestered by each tree species, and for comparison purposes, the tree density was kept at 111 trees ha^{-1}; common density for hardwood tree species grown in TBI systems in the temperate region (Thevathasan and Gordon 2004).

Table 1. Carbon sequestration (t C ha^{-1} y^{-1}) potentials of five tree species commonly grown in tree-based intercropping systems in comparison to conventional agricultural systems in southern Ontario, Canada

Inputs	Poplar	Oak	Walnut	Spruce	Cedar	Soybean Monocrop
Aboveground tree C assimilation	0.83	0.46	0.48	0.38	0.53	
Belowground tree C assimilation	0.23	0.16	0.11	0.14	0.12	
Litterfall C inputs	1.63	1.07	1.50	1.49	0.68	
Fine root turnover	0.82	0.54	0.75	0.45	0.20	
Above and belowground Crop C input	1.22	1.22	1.22	1.22	1.22	1.40
Outputs (via decomposition)						
Litterfall C outputs	1.04	0.54	1.44	0.63	0.26	0
Root output	0.52	0.27	0.72	0.19	0.08	1.31
Crop C outputs	1.00	1.00	1.00	1.00	1.00	1.19
C leachate	0.05	0.05	0.05	0.04	0.04	0.05
Net						
Net C balance	+ 2.12	+ 1.58	+ 0.84	+ 1.81	+ 1.36	- 1.15

Results related to the abundance of key functional genes in the nitrification and denitrification pathways showed that both tree species and proximity to the tree, can influence the abundance of key microbial groups associated with N_2O production, particularly organisms associated with denitrification, nosZ and nirS (**Figure 2a and b**).
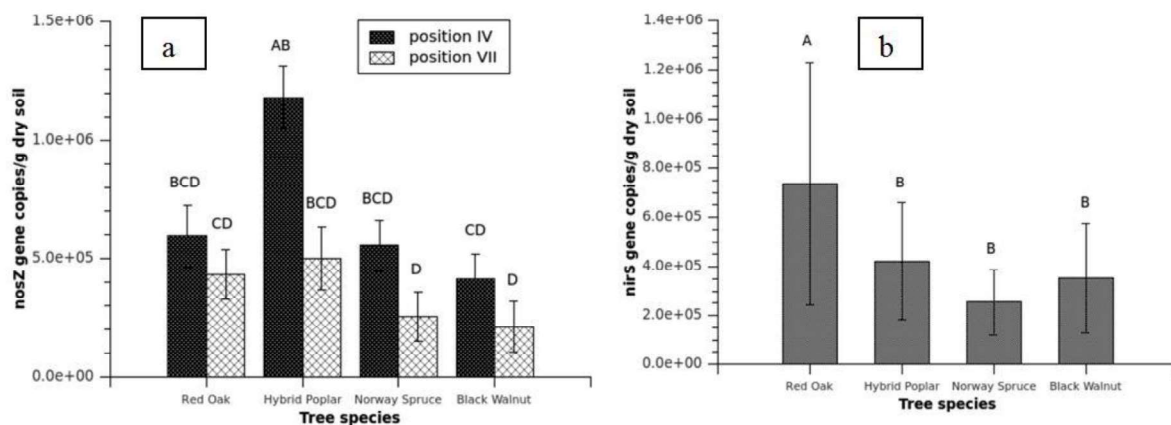
Figure 2a and b. a: Mean abundance of nosZ genes found for transect positions IV (tree row) and VII (6 m into the copping alley), and b: nirS gene across all transect positions, for each tree species tested. Bars marked with the same letter within the same graph are not significantly different (p > 0.05).

Oak and poplar showed different N_2O emission potential, with poplar having higher accumulation within the modified denitrification assay that was performed. This assay interrupts the complete denitrification of nitrate at the point of the cycle mediated by nitrous oxide reductase, the enzyme coded for by nosZ. It was interesting that blocking the action of nosZ in oak soil (which had a greater abundance of the nirS gene) did not result in greater accumulation of N_2O from the oak soil, when compared to the poplar soil. While these results do support the assertion that tree species selection may influence N_2O emissions from agroforestry systems, this study was conducted using soil from only one site (as described above). TBI systems can be found across the globe, with huge differences in climate, soil structure, and choice of crop. It is not possible, from this study, to assert that in all environments, under all management practices, these same tree-gene trends will exist. Further work needs to be done to confirm that different crop types, time of year, or long term fertilization treatments do not assert an equal or greater influence on the soil microbial community structure in different locations, before any recommendation of specific tree species can be made that are capable of contributing to N_2O mitigation via microbial populations.

Denitrification, which significantly contributes to N_2O production, is driven to a large extent by soil oxygen levels, which themselves are largely determined by a soil's intra-aggregate void space. Soils under poplar had a significantly ($p < 0.05$) greater proportion of small intra-aggregate voids than soils under spruce (**Figure 3**). The most cited tree species effect on soils is the variability in tree leaf litter quantity, and chemical inputs to surrounding soils (Binkley 1996). Wotherspoon et al., (2014) studying the same TBI system determined by in situ measurements that highest litterfall was found beneath poplar trees, followed by walnut, spruce, and oak with 3.79, 3.48, 2.97, and 2.50 t ha^{-1} y[-1], respectively. It was also determined that the rate of litter decomposition varied between tree species; Walnut litter decomposition proceeded most quickly, followed by poplar and oak, while spruce litter decomposed the slowest due, to the presence of varying amounts of lignin present in the litter. The highest amount of leaf-litter input measured under poplar and the lowest decomposition rate found in spruce might have contributed towards higher small intra-aggregate voids in soil under poplar. Differences in void spaces in soils under tree-based intercropping systems could also influence denitrification rates and N_2O emissions from these systems, as indicated above.
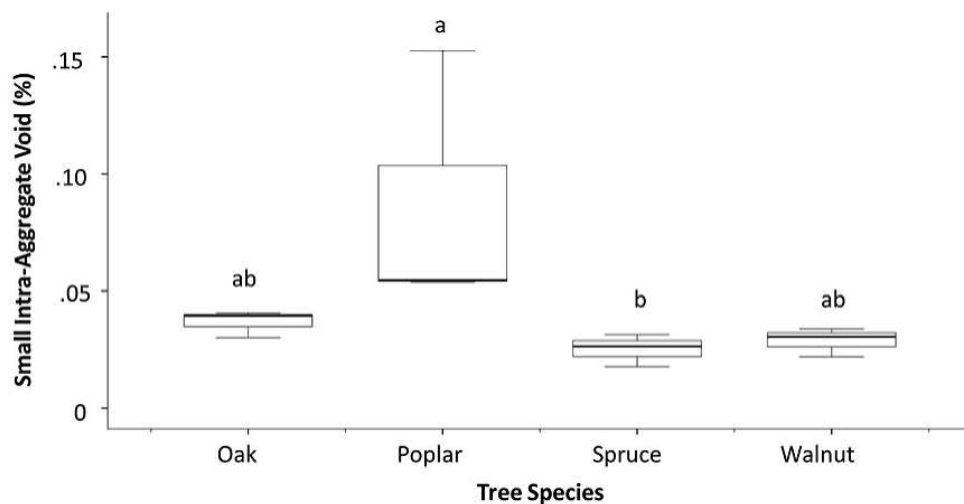
Figure 3. Small Intra-aggregate void space as a percentage of solid 'aggregate' volume identified by X-ray µCT for four tree species. Bars show max and min, box represents 25th -75th percentile, and middle line represents median value (n=3). Bars labelled with the same letters were not significantly different according to Kruskal-Wallis test and Nemenyi-Dunn Post hoc test.

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