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Agroforestry promotes soybean yield stability and N$_2$-fixation under water stress

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Abstract Agroforestry systems can buffer crop performance against the impacts of climate change, particularly decreases in the availability of soil water. Nonetheless, farmers are reluctant to adopt agroforestry systems due to concerns of yield losses, predominantly in the tree-crop competitive zone. Yet little is known about crop performance in the tree-crop competitive zone under water limited conditions. We therefore studied the effect of a full season water deficit on soybean N$_2$-fixation, nodulation, and yield in the tree-crop zone of a mature agroforestry system. We hypothesized higher N$_2$-fixation yet lower but stable yield in the tree-crop zone. Rainfall reduction shelters were used to reduce available soil moisture throughout the growing season in the tree-crop competitive zone of a 27-year-old tree-based intercropping agroforestry system and a paired monoculture control plot in southern Ontario, Canada. Results show that soybean yields in the tree-crop zone were lower compared to monoculture. However, soybean yields were stable in agroforestry and only in monoculture did the rainfall reduction induce a significant decline in soybean yields. Soybeans in the tree-crop zone relied heavily on N$_2$-fixation to meet N demand, with a percentage of N derived from atmosphere (%Ndfa) of 91 % versus 63 % in monoculture. However, total fixed N declined significantly under rainfall reduction in both the tree-crop zone and in monoculture. Of note, soybean nodulation patterns adapted to soil moisture availability, allocating a larger proportion of nodules lower on the rooting system under water limitation. Our results demonstrate that important N pathways may be altered under water limitation. We can also expect that in areas where growing conditions are predicted to become drier in the future, yields in the tree-crop competitive zone will not be reduced further, thus increasing the viability of adopting agroforestry systems in areas affected by climatic change.

Keywords Climate change · ^{15}N natural abundance method · Nodulation · Tree-based intercropping · Water stress

1 Introduction

In several major soybean growing regions, rainfall is predicted to become less evenly distributed over the growing season, while increases in temperatures and evapotranspiration will limit the availability of adequate soil moisture (Piao et al. 2010; Hatfield et al. 2014). Already, recent trends in precipitation and temperature since 1980 have detrimentally impacted soybean yields in the largely non-irrigated upper Midwest of the USA (Mourtzinis et al. 2015). Soybean yields are particularly sensitive to water deficit, with even mild or moderate water deficits (i.e., no obvious visible signs of water stress such as wilting) resulting in significant yield reductions (Desclaux et al. 2000; Purcell et al. 2004). N$_2$-fixation in soybean is another process which is notoriously sensitive to water deficit (Serraj et al. 1999). While across a variety of cropping systems and growing conditions, N$_2$-fixation supplies approximately 50–60 % of total soybean N demand (Salvagiotti et al. 2008), N$_2$-fixation rates are only maximized at field capacity

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(Djekoun and Planchon 1991) and decline with even small decreases in soil moisture (Hungria and Vargas 2000).

The integration of trees into agricultural landscapes, or agroforestry, represents a potential agricultural adaptation measure that may help buffer soybean performance against predicted changes in growing conditions. Agroforestry systems have been found to buffer the effect of extreme weather on farm-field microclimate by reducing fluctuations in both air and soil temperatures, humidity and by reducing wind velocity near the soil surface (Lin 2007; Böhm et al. 2014; van Noordwijk et al. 2014). In general, microclimatic modifications in agroforestry systems also reduce soil moisture evaporation (Siriri et al. 2013) while other authors have found that agroforestry systems increased infiltration and soil water storage (Anderson et al. 2009). Near the tree row, however, competition for soil moisture may limit the availability of water for crop use early in the growing season (Link et al. 2015). Several authors have recommended agroforestry systems for water deficient regions or as an adaptation measure for future predicted decreases in soil moisture availability (Lin 2007; Sileshi et al. 2012) as yields tend to be more stable across environmental conditions in these modified landscapes.

Although such results are promising, adoption of agroforestry systems, such as tree-based intercropping systems, in temperate regions is constrained by farmer concerns of crop losses in the tree-crop competitive zone (Valdivia et al. 2012), an area extending several meters into the crop alley in which tree-crop competition limits crop yield (Thevathasan and Gordon 2004). Although several authors have found that outside of the tree-crop competitive zone, soybean yields in agroforestry systems are equal to or higher than in soybean monoculture (Guto et al. 2012; Reynolds et al. 2007), in mature agroforestry systems the competitive zone can represent a non-trivial portion of the total cropped area (Thevathasan and Gordon 2004).

The objective of this study was to examine the effect of a full season water deficit in the field on soybean N_2-fixation, nodulation, and yield in the tree-crop competitive zone of an agroforestry system. To do this, we simulated a reduction in available soil moisture throughout the growing season using rainfall reduction shelters in a 27-year-old tree-based intercropping agroforestry system and a paired monoculture control. We quantified soybean biomass, nodulation, and N_2-fixation using the ^{15}N natural abundance method at three different soybean growth stages in addition to seed yield at harvest. We hypothesized that rainfall reduction will limit soybean N_2-fixation compared to full rainfall but that the relative magnitude of this limitation will be smaller in the tree-crop competitive zone of the agroforestry system as compared to in the monoculture. Furthermore, although soybean performance may be suppressed in the tree-crop competitive zone of the agroforestry system as compared to

monoculture, yield stability will be more readily apparent under rainfall reduction.

2 Materials and methods

2.1 Site description

The experiment was conducted at the University of Guelph Agroforestry Research Station (43°32′49 N, 80°12′44 W, elevation 325 m), Guelph, Ontario, Canada. The 30-ha site houses both a 27-year-old tree-based intercropping system, with 17 tree species planted randomly (in blocks of eight trees) in rows either 12.5 or 15 m apart, as well as adjacent open fields (Thevathasan and Gordon 2004). The soil is classified as a Typic Hapludalf with a sandy loam texture (Thevathasan and Gordon 2004). During the experiment, the average weekly maximum temperature was 27.3 °C and the average weekly minimum temperature was 6.0 °C (1550 growing degree days). Monthly mean temperatures during the growing season were never more than ±1.3 °C long-term monthly means (1981–2010). Higher than average rainfall compared to historical average (1980–2010) occurred in July (157 vs. 89 mm) and September (200 vs. 93 mm), while lower than historical average rainfall occurred in June (66 vs. 83 mm) and August (65 vs. 97 mm). Current and historical weather data was collected from a weather station 28 km from the experimental site.

2.2 Experimental design

The experimental design was a split-plot design with two planting regimes, monoculture and tree-based intercropping, as the main whole plot effect, replicated in four blocks. Split-plot effects consisted of two randomly assigned treatments of full rainfall or rainfall reduction. To simulate a full season reduction in rainfall and induce a water deficit, rainfall reduction shelters, based on the design of Yahdjian and Sala (2002), were placed 1 week after soybean emergence (Fig. 1). These fixed location structures use v-shaped acrylic troughs to intercept and divert rainfall off of the area beneath the shelter, which measured 2.5×1.1 m (2.75 m²) in this experiment. All samples were taken at least 20 cm away from the shelter perimeter to avoid the impact of lateral water flow from adjacent areas into the rainfall reduction shelters. In the tree-based intercropping system, split-plots (full rainfall and rainfall reduction) were located 2 m away from the tree row, directly in front of a tree trunk, and thus within the canopy drip zone. Of the tree species present in the tree-based intercropping system, *Acer saccharinum* (silver maple), a tree species recommended for windbreaks across a variety of Ontario soil types (Ontario Ministry of Natural Resources 1995), was selected as the alley row tree species within this experiment. In silver maples, both
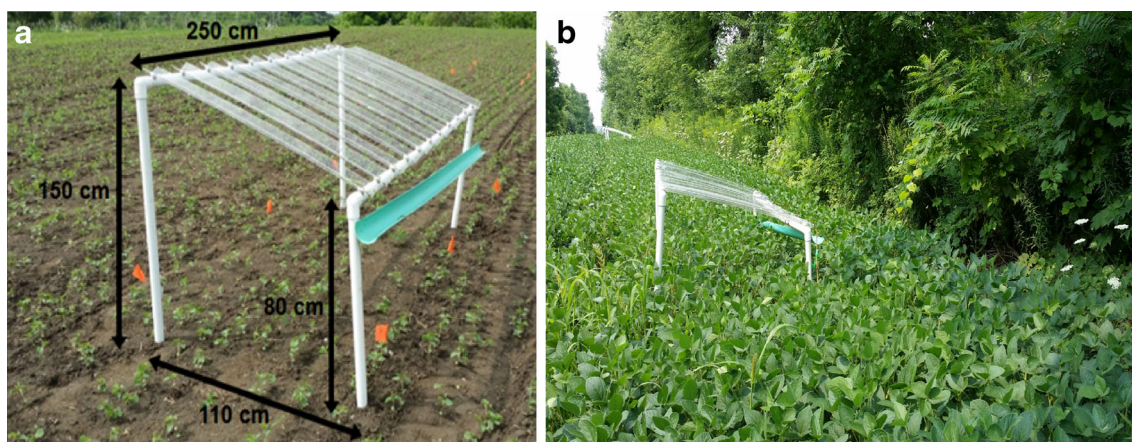
Fig. 1 Rainfall shelters, based on the design of Yahdjian and Sala (2002), in **a** monoculture and **b** the tree-crop competitive zone of an agroforestry system. PVC tubing was used for shelter frame and clear acrylic for the troughs. Total shelter footprint was 2.75 m^2 (1.1×2.5 m)

trunk and branches are mixed orthotropic sympodia that are rhythmically branched, while lateral shoots are oblique monopodia which are also rhythmically branched; tree crown is generally broad and rounded (Millet and Bouchard 2003). Mean tree height in this experiment was approximately 15.5 (±0.90) m.

Air temperature, relative humidity, photosynthetically active radiation (PAR), and volumetric soil moisture (top 10 cm of soil profile) were monitored by eight data loggers (Onset HOBO®—USA), with four in each whole plot treatment and two in each split-plot treatment, taking measurements at 30-min intervals throughout the growing season (Table 1). Mean weekly volumetric soil moisture in both tree-based intercropping and monoculture systems, averaged across the rainfall reduction and full rainfall treatments, is shown in Fig. 2. Differences in volumetric soil moisture across rainfall treatments varied throughout the growing season, though on average soil moisture under the rainfall reduction treatments was reduced by 12.8 and 16.2 % in monoculture and tree-based intercropping systems, respectively (Table 1). The Ontario Ministry of Natural Resources (as cited in Environmental Commission of Ontario 2008) estimates that in the future, southern Ontario will receive on average 10–20 % less precipitation during the growing season, suggesting that on average volumetric soil moisture in the rainfall reduction treatment is broadly representative of future predicted growing conditions in southern Ontario (Table 1; Fig. 2).

Soybean (variety, Pioneer P90Y90; crop heat units=2750) was planted at a seeding rate of roughly 450,000 seeds ha^{-1} (7.5 in. row spacing) in both the tree-based intercropping and monoculture system. A soybean plant density check mid-way through the season revealed no significant differences in plant density across treatments. Soil testing at planting revealed no major soil nutrient deficiencies and no fertilizer was applied. Hand weeding was performed throughout the growing season to control weeds in experimental plots.

2.3 Plant and soil sampling

Plants and soil were sampled together on three sampling dates throughout the growing season: at the V5 (vegetative) stage, the R3 (beginning pod) stage, and the R6 (seed-filling) stage of reproductive growth (Fehr et al. 1971). On each sampling date, in each split-plot, a 25-cm^2 quadrat containing three soybeans was randomly selected and three whole soybean plants along with entire intact root system and nodules were excavated from the area, capturing lateral roots in the soil profile. Since the vast majority of nodules are located within the top 10 to 15 cm of the soil profile (Hardarson et al. 1989; Shimada et al. 2012), this approach had a high likelihood of accurately measuring nodulation patterns. Additionally, a soil sample from the same quadrat was collected with a 2-cm-diameter soil core to a depth of 20 cm.

Soybean plants were washed in distilled water to remove soil, and the soybean root system (with nodules) was separated from the shoot. The washed whole soybean root system was divided into an upper half and a lower half, using the midway point between the first and last lateral root on the taproot to demarcate the upper root system from the lower root system, following Bargaz et al. (2015). The number of nodules on each half of the root system was counted to determine nodule dry weight (NDW). Soybean shoots and nodules were then dried at 65 °C for 24 h, after which dry masses for all plant parts were immediately recorded. Final yield determination (pod number and seed weight) occurred at R8, selecting three soybean plants in a 25-cm^2 quadrat. Seeds were separated from pods, dried at 65 °C for 24 h, and weighed immediately afterwards.

Soil samples were divided into three subsamples for determination of gravimetric soil moisture content (%) and plant-available N (mg NO$_3^-$ kg^{-1} and mg NH$_4^+$ kg^{-1}) on fresh samples and available phosphorus (P) (mg kg^{-1}) on air dried samples. Plant-available N was determined from 2 g of soil

Table 1 Means (±standard error) for volumetric soil moisture (m^3 m^{-3}), relative humidity (%), temperature (°C), and photosynthetically active radiation (PAR) (μ mol m^{-2} s^{-1}) registered by data loggers during 14 weeks of growth across full rainfall and rainfall reduction treatments in both monoculture and the tree-crop competitive zone of an agroforestry system (the calculated difference (%) in these parameters across rainfall treatments is also shown)

	Monoculture			Agroforestry system		
	Full rainfall	Rainfall reduction	Percent difference (%)	Full rainfall	Rainfall reduction	Percent difference (%)
Soil moisture	0.220±0.006	0.195±0.007	12.8	0.179±0.007	0.154±0.009	16.2
Relative humidity	86.8±0.7	86.6±0.1	0.2	86.5±4.0	84.1±1.0	−2.8
Temperature	17.5±0.4	17.6±0.4	−0.6	17.7±0.4	18.2±0.4	−2.7
PAR	529±46	488±42	8.4	222±14	243±20	−8.6

extracted with 20 mL of potassium chloride (KCl). Available soil P determined with 4 g of soil was extracted using 20 mL of Bray-1 solution. These extractions were then used to determine plant-available N and plant-available P colorimetrically using flow injection analysis (Lachat QuikChem; Lachat Instruments, Colorado, USA). We found no significant differences in plant-available N or P across plots or rainfall treatments (data not shown).

2.3.1 N_2-fixation and fixed N quantification

N_2-fixation was estimated using the ^{15}N natural abundance method (Mariotti et al. 1981), a method that has been used in previous studies at the Guelph Agroforestry Research Station (Oelbermann and Voroney 2007; Isaac et al. 2014). A composite sample was formed from the quadrat of each split-plot on each sampling date by grinding aboveground non-reproductive soybean biomass (i.e., shoots and leaves) in a ball mill (Retsch/Hann—Germany). Each composite sample was analyzed for N concentration and ^{15}N/^{14}N ratio by dry combustion in an elemental analyzer (CHNS-O EA1108—Italy) coupled to a Delta[plus] XL continuous-flow stable isotope ratio mass spectrometer (Thermo Finnigan/Bremen—Germany) at the University of Waterloo Environmental Isotopes Laboratory in Waterloo, Canada. The $\delta^{15}N$ (‰) of the sample was calculated using Eq. 1, following Mariotti et al. (1981):

$$\delta^{15}N(‰) = \left(\frac{R_{sample}}{R_{air}-1}\right) \times 1000 \qquad (1)$$
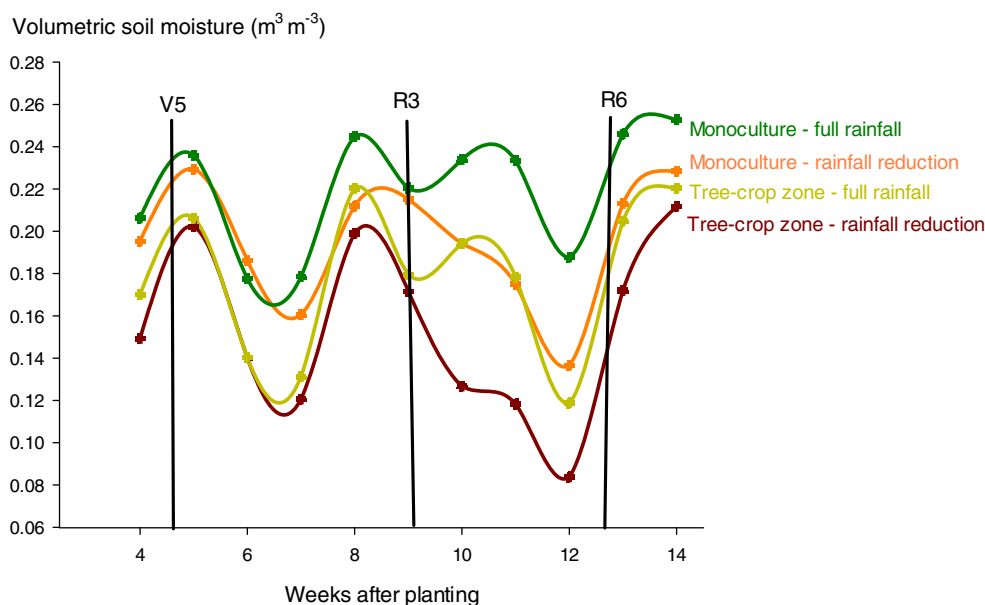
Fig. 2 Mean weekly volumetric soil moisture (m^3 m^{-3}) content (top 10 cm) in full rainfall and rainfall reduction treatments across the tree-crop competitive zone of an agroforestry system and in monoculture from 4 to 14 weeks after soybean planting. All data is taken from HOBO data loggers. All three sampling times are shown along with the developmental stage of the soybean (V5, R3, and R6 stage of development) at sampling time. Across whole plots, volumetric soil moisture is lower in the rainfall reduction treatment compared to full rainfall. This difference in volumetric soil moisture is largest between the R3 and R6 stages of soybean reproductive development, when soybean yield components are known to be affected by water deficit

where R denotes the $^{15}N/^{14}N$ ratio of the sample and air (0.3663‰), respectively. Percentage of N derived from atmosphere (%Ndfa) was calculated using Eq. 2, as follows:

$$\%\text{Ndfa} = \left[\frac{\left(\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_x \right)}{\left(\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_B \right)} \right] \times 100 \qquad (2)$$

where $\delta^{15}\text{N}_{\text{ref}}$ is the $\delta^{15}\text{N}$ value of the reference plant, $\delta^{15}\text{N}_B$ is the $\delta^{15}\text{N}$ of soybean deriving N exclusively from N_2-fixation, and $\delta^{15}\text{N}_x$ is the $\delta^{15}\text{N}$ value of the sample legume under study.

Reference values were determined with non-nodulating soybeans grown in both the monoculture and tree-based intercropping system in 2.5×1.1 m plots in close proximity (less than 20 m) to the experimental split-plots. Reference plants were sampled on the same three dates as the soybeans under study, with a 25-cm^2 quadrat used to select non-nodulating soybeans in both the monoculture and tree-based intercropping systems. Soybean samples were matched to reference plants (i.e., non-nodulating soybean) from the same management regime (monoculture or tree-based intercropping) and the same sampling date. Mean $\delta^{15}\text{N}_{\text{ref}}$ values declined over the course of growing season, declining from 2.41‰ (V5) to 2.06‰ (R3) to 0.90‰ (R6) in monoculture and from 2.97‰ (V5) to 1.65‰ (R3) to 0.90‰ (R6) in the competitive zone of the tree-based intercropping system. $\delta^{15}\text{N}_B$, also known as the B value, was determined by growing soybeans under study in a greenhouse hydroponics system with an N-free nutrient solution and inoculated with *Bradyrhizobium japonicum*. Soybeans were harvested 7 weeks after germination at the full bloom stage (R2) of development. Three soybeans shoots were dried and ground to form a composite sample. The three replicates were sent for $\delta^{15}\text{N}_B$ determination, and the $\delta^{15}\text{N}_B$ used in this study was the lowest $\delta^{15}\text{N}_B$ recorded.

Total fixed N in soybeans was calculated as the product of mean shoot N concentration, %Ndfa, and shoot weight. Partial N balance, which provides a simple overview of farm field N inflows and outflows at harvest, was calculated following Salvagiotti et al. (2008), with fixed N measured at R6 and seed N measured at R8, assuming a mean seed N concentration of 6.34 % (Salvagiotti et al. 2008). Partial N balance was calculated as the difference between fixed N in aboveground biomass (shoot weight × N concentration × %Ndfa) and total N in harvested seeds (seed weight × 6.34 %); negative values represent larger net farm field N losses while positive values represent farm field N gains.

2.4 Statistical analysis

All data analyses were conducted in the R v.3.0.2 (R Foundation for Statistical Computing—Vienna, Austria). Prior to all analyses, dependent variables were assessed for normality with the Shapiro-Wilk test; the type I error rate of these tests was set at 0.05. If assumptions were not met, dependent variables were transformed based on the Box-Cox method (MASS package) and again tested for normality. The exception to this was shoot N concentration and the proportion of nodules on the bottom root half, which was transformed using a logistic function to achieve normality. All post hoc analysis consisted of a pairwise multiple comparison using Tukey's HSD test (multcomp package).

The statistical procedure used to model continuous variables measured on one sampling date as a function of management regime and rainfall treatment was a mixed-effect split-plot ANOVA with an error term that treated block effects as random (aov function). For variables measured at all three sampling times, a repeated measures ANOVA was used (aov function). The mean quadrat value was used for all morphological traits and yield components ($n=4$) while for %Ndfa, shoot N concentration, and total fixed N, a smaller sample size was used ($n=3$).

3 Results and discussion

3.1 Impact of management regime and water deficit on N_2-fixation, nodulation, and field N cycling

The final estimate of %Ndfa in monoculture during seed filling (R6) revealed that slightly more than 60 % of soybean N demand was met by N_2-fixation, averaged across rainfall treatments (Table 2), a result which is in line with the findings of a previous review of North American soybean N_2-fixation studies (Salvagiotti et al. 2008). Furthermore, estimates of soybean N_2-fixation in the monoculture plot were in line with findings of previous work studying the time course and duration of N_2-fixation (Zapata et al. 1987), with N_2-fixation rates increasing significantly ($p=0.009$) between vegetative and reproductive development. The significant increase in %Ndfa between vegetative and specifically early pod development (R3) coincided with a significant ($p<0.001$) increase in NDW (from 110 ± 33 mg plant^{-1} to 663 ± 102 mg plant^{-1} in full rainfall and from 86 ± 12 mg plant^{-1} to 459 ± 36 mg plant^{-1} in rainfall reduction) (Fig. 3b).

In the tree-crop competitive zone of the tree-based intercrop, %Ndfa exhibited a different pattern over the growing season as soybeans shifted N acquisition strategies. At all three soybean growth stages, mean %Ndfa in soybean was significantly ($p<0.001$) higher in the tree-crop competitive zone compared to monoculture, irrespective of rainfall treatment (Table 2). At the V5 stage of vegetative growth, over 60 % of soybean N demand was met by N_2-fixation; this is an astonishingly high %Ndfa value for soybeans still in the stages of vegetative growth (Zapata et al. 1987). Intraspecific competition for soil N may explain the augmented N_2-fixation of soybeans in the agroforestry system, as both Rivest et al.

Table 2 Means (±standard error) of N derived from atmosphere (Ndfa) (%), total shoot N concentration (%), and fixed N (mg N plant^{-1}) for soybeans grown in the tree-crop competitive zone of an agroforestry system and in monoculture plots across both full rainfall and rainfall reduction treatments on all three sampling dates

	Monoculture		Agroforestry system	
	Full rainfall	Rainfall reduction	Full rainfall	Rainfall reduction
%Ndfa				
V5 (vegetative)	2.7±0.88 ax	9.0±4.62 ax	64.3±4.10 bx	65.0±1.15 bx
R3 (beginning pod)	49.7±7.97 ay	43.7±2.85 ay	77.0±4.04 bx	77.7±4.84 bx
R6 (seed fill)	65.0±8.00 ay	61.3±2.60 ay	94.3±4.66 by	88.3±6.69 by
Shoot N concentration				
V5 (vegetative)	3.6±0.13 ax	3.3±0.11 ax	2.9±0.10 ax	2.8±0.44 ax
R3 (beginning pod)	2.8±0.20 abx	2.5±0.13 abx	2.2±0.19 ax	2.1±0.09 ax
R6 (seed fill)	1.8±0.35 bx	1.8±0.34 bx	1.1±0.24 by	0.8±0.12 by
Fixed N				
V5 (vegetative)	3.2±1.17 ax	8.8±4.70 ax	14.6±1.22 ax	27.9±13.05 ax
R3 (beginning pod)	120.4±27.27 ay	82.2±3.46 ay	79.7±5.49 abx	49.9±6.34 bx
R6 (seed fill)	121.0±11.66 ay	97.7±26.99 ay	40.6±12.61 bx	20.9±9.76 bx

Means within a row followed by different letters (a–b) are significantly different at $p < 0.05$ ($n=3$). Means within a column for each parameter followed by different letters (x–y) are significantly different at $p < 0.05$ ($n=3$)

(2009) and Isaac et al. (2014) hypothesized. Link et al. (2015), in a study at the same site, found active fine tree roots in the top 20 cm of the soil in May, suggesting that competition for soil N begins early in the growing season.

Total fixed N was significantly ($p=0.017$) lower at the R6 stage of development under the rainfall reduction treatment compared to the full rainfall treatment in both monoculture (97.7 vs. 121.0 mg N plant^{-1}) and the tree-crop competitive zone (20.9 vs. 40.6 mg N plant^{-1}) (Table 2). Given that total fixed N is a product of biomass, %Ndfa, and shoot N concentration, this occurred because soybeans grown under rainfall reduction tended to have slightly lower shoot biomass values compared to soybeans under full rainfall (Table 2). When aggregated to calculate total fixed N, differences in these three variables across rainfall treatments drove the divergence in total fixed N across treatments. Similarly, total fixed N in monoculture was significantly ($p=0.002$) higher as compared to the tree-crop competitive zone, despite higher mean %Ndfa values; larger mean shoot weights and N concentrations in monoculture translated into higher total fixed N, on average, compared to soybeans in the tree-crop competitive zone (Table 2).

Nodule dry weight (NDW) was also significantly ($p=0.004$) different in monoculture compared to the tree-crop competitive zone across rainfall treatments at R3 and R6, with lower NDW associated with the tree-crop competitive zone. Soybean nodulation patterns differed across all treatments. Soybeans allocated a significantly ($p=0.013$) larger proportion of NDW to the lower root system in the tree-crop competitive zone (64.3 %) compared to monoculture (48.8 %) (Fig. 3c). Additionally, a strong trend in the proportion of NDW allocated to the lower root system was found across rainfall treatments, being greater in the rainfall reduction treatment compared to the full rainfall

treatment in both plots on all sampling dates (Fig. 3c). Results from our experiment suggest that soybean nodulation pattern is influenced by the availability of soil moisture as well as by intraspecific competition. In the tree-crop competitive zone, where volumetric soil moisture in the top 10 cm was lower compared to monoculture (Table 1; Fig. 2), and in the rainfall reduction treatment of both plots compared to full rainfall (Table 1; Fig. 2), soybean allocated a larger proportion of NDW to the lower root system (Fig. 3). Previously, Shimada et al. (2012) found that soybeans allocated significantly more nodules deeper in the soil layer as the depth of the water table increased, while Hardarson et al. (1989) found that soybeans with the nodules deeper in the soil profile (10–15 cm) contributed more to N$_2$-fixation than shallower nodules (0–10 cm). Nodule N$_2$-fixing ability is known to be closely related to nodule water potential and nodule water content, with dry soil desiccating nodules, interfering with regulation of nodule O$_2$ diffusion and temporarily preventing N$_2$-fixation (Djekoun and Planchon 1991; Fleurat-Lessard et al. 2005). This suggests that if soybean nodules are allocated preferentially several centimeters deeper in the soil profile, where more soil moisture is presumably available, N$_2$-fixation could be maintained or buffered against mild or moderate water deficits. These findings suggest an interesting morphological adaptation measure of soybeans that may be further exploited to maintain N$_2$-fixation potential in superficially dry soils.

Results of this study suggest that in areas where soybean is grown, limitations on soil moisture may reduce the total amount of N fixed by soybeans and thus the annual amount of N being introduced into N cycles. However, partial N balance was more negative—meaning more N was removed from the farm field after harvest—under full rainfall compared to rainfall reduction in both monoculture

Fig. 3 Means (±SE) **a** shoot dry weight (g plant^{-1}), **b** nodule dry weight ▶
(g plant^{-1}), and **c** the proportion of nodule dry weight on the lower root
system for soybean grown in the tree-crop competitive zone of a
agroforestry system and in monoculture plots across full rainfall and
rainfall reduction treatments on three sampling dates ($n=4$). Across all
treatments, shoot dry weight (**a**) increased significantly between V5 stage
of development and R3 stage of development only, while nodule dry
weight increased significantly across all three sampling dates (V5, R3,
and R6 stage of development). Shoot dry weight (**a**) and nodule dry
weight (**b**) were significantly larger in monoculture compared to the
tree-crop competitive zone on all sampling dates. The proportion of
nodule dry weight on the lower root system (**c**) was significantly larger
in the tree-crop competitive zone compared to monoculture, and generally
larger in the rainfall reduction treatments compared to full rainfall

(-190 ± 78.6 kg N ha^{-1} in full rainfall vs. $-147\pm$
54.4 kg N ha^{-1} in rainfall reduction) and the tree-crop
competitive zone (-108 ± 47.9 kg N ha^{-1} in full rainfall
vs. -89 ± 21.5 kg N ha^{-1} in rainfall reduction). Although
total fixed N was higher under full rainfall in both mono-
culture and in the tree-crop competitive zone, soybean
seed yield, which is relatively high in N content and re-
moved from the field after harvest, was large enough un-
der full rainfall to offset the increase in fixed N. Partial N
balance was largest in the tree-crop competitive zone com-
pared to monoculture across both rainfall treatments, sug-
gesting that N cycling is enhanced in the tree-crop com-
petitive zone, especially under water deficit.

3.2 Yield and yield component response to water deficit and management regime

Water deficit is known to impact soybean seed yield, though
the magnitude of this impact and the soybean yield compo-
nent(s) affected depends on the soybean growth stage at which
water deficit is experienced (Desclaux et al. 2000). In mono-
culture, seed yield decreased significantly ($p=0.012$) under
rainfall reduction compared to full rainfall, declining by over
40 % (from 294 ± 59.0 g m^{-2} in full rainfall to 200 ± 45.6 g m^{-2}
under rainfall reduction). No significant differences were ob-
served in mean soybean seed yield across rainfall treatments
in the tree-crop competitive zone (106 ± 16.2 g m^{-2} in full
rainfall to 88 ± 10.27 g m^{-2} in rainfall reduction). Across man-
agement treatments, seed yield was significantly lower in the
tree-crop competitive zone ($p<0.001$) compared to monocul-
ture, with yields in monoculture roughly double those in the
tree-crop competitive zone. These results are unsurprising giv-
en competition from the tree row for above- and belowground
resources, which may explain the significantly lower soybean
yields observed in the tree-crop competitive zone compared to
the monoculture plot (Reynolds et al. 2007; Isaac et al. 2014).
However, our results demonstrate that yields in the tree-crop
competitive zone are stable across soil moisture regimes and
decline less compared to monoculture under rainfall reduc-
tion. Mechanistically, this finding may be explained by the
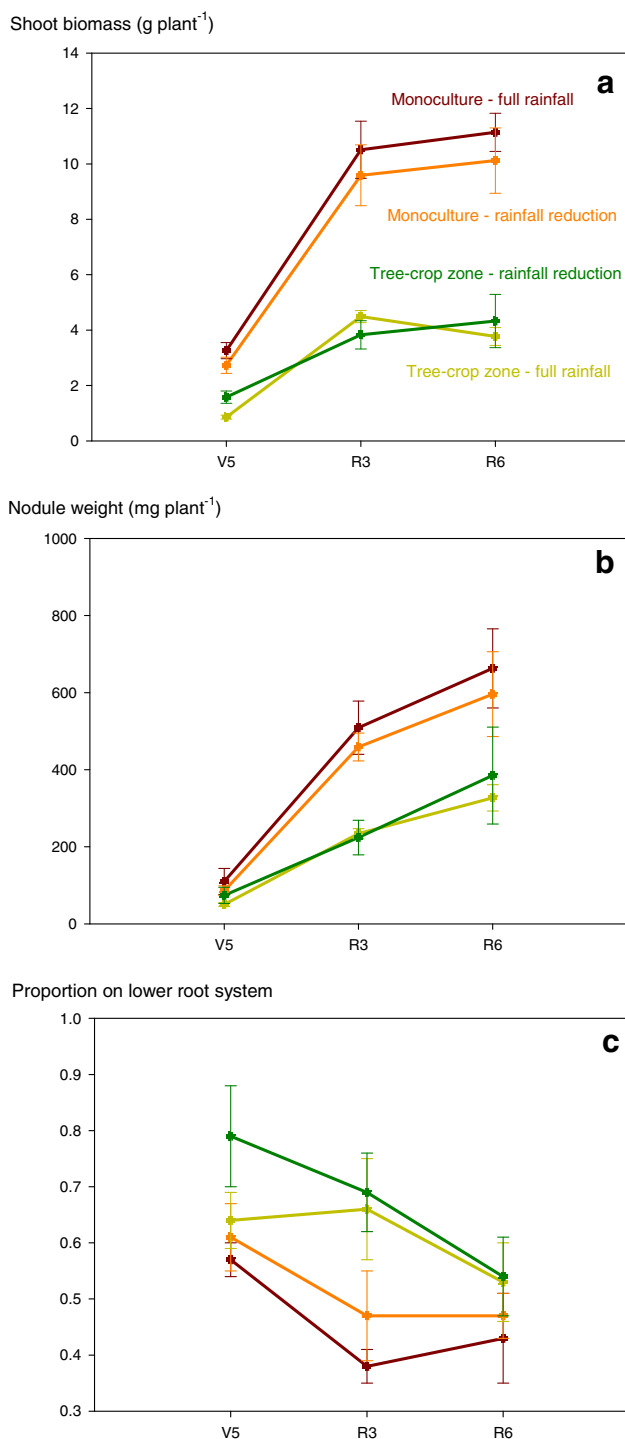
various modifications imposed by trees on the microclimate
(Siriri et al. 2013; Böhm et al. 2014), soil physical properties
(Thevathasan and Gordon 2004; Sileshi et al. 2012), soil bio-
diversity (Bainard et al. 2011), infiltration, and soil water hold-
ing capacity (Verchot et al. 2007) of agroforestry systems. Al-
though competition for soil moisture near the tree row may limit
the availability of water for crop use (Reynolds et al. 2007),

some studies note water use complementarity between crops and the tree row (Chirwa et al. 2007; Guto et al. 2012), while Link et al. (2015) found that trees in the tree row shift water acquisition below the crop root zone. Our findings support previous work studying yield stability in agroforestry systems (Sileshi et al. 2012; Guto et al. 2012) across growing conditions and suggest that as soil moisture availability in soybean growing regions declines in the future, the yield gap between monoculture and the tree-crop competitive zone may narrow.

4 Conclusion

Soybean production in many areas of the world has already been detrimentally affected by changes in temperature and precipitation patterns, a problem which will be exacerbated in the future as climate change reduces the reliability and availability of soil moisture in certain areas. We find that soybean nodulation patterns respond to reduced soil moisture in a way that may buffer N_2-fixation capacity against water deficit. We also show that the yield gap between soybean grown in monoculture and soybean grown in agroforestry systems decreases under soil water limitation, and that yield stability is prevalent across a moisture deficit in the tree-crop competitive zone of an agroforestry system. Taken together, we illustrate that as soil moisture availability declines in the future, agroforestry systems such as tree-based intercrops will become increasingly viable cropping systems for soybean production.

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