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RESEARCH PAPER

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Effects of Root Interaction and Nitrogen Fertilization on the Chlorophyll Content, Root Activity, Photosynthetic Characteristics of Intercropped Soybean and Microbial Quantity in the Rhizosphere

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ABSTRACT

Nitrogen is the most important fertilizer element in cranberry production determining vegetative growth and productivity. Your choice of N rates and timing can make the difference between adequate growth and high yields and excessive growth with poor cropping. Choice of N form can be important in maximizing the efficiency of fertilizer use and in protecting environmental quality. These factors will be discussed and factors affecting N fertilizer use will be explored. N rates have been studied in several growing areas and on various cultivars. A common result in these studies was the observation that no treatment effect is apparent in the first year of the study. That is, plots receiving no fertilizer had similar yield to any of the N rate plots. This is evidence for the theory that fertilizer applied this season has little effect on this crop but rather is important for next year. Root interaction and increasing nitrogen application improved soybean yield and its components, but their effects gradually decreased with increasing nitrogen fertilization level. The root activity of soybean was obviously affected by root interaction, and was significantly positively correlated with green leaf area per plant, chlorophyll content, photosynthetic rate and economic yield per plant. Our results indicate that the advantage effect of root interaction and increasing nitrogen application will be partially inhibited with an increasing nitrogen fertilization level.
Keywords: Gas-Exchange Parameter, Nitrogen Nutrition, Plant Pigments and Root Separation.

INTRODUCTION

Facilitation takes place when plants ameliorate the environment of their neighbors, and increase their growth and survival (Hauggaard-Nielsen and Jensen 2005). Yields of intercrops may exceed the yield sum of the component species grown alone, as a result of better use of available growth resources such as nutrients, water, and light (Miyazawa et al. 2010). The study by Li et al. (1999) showed that total biomass and grain yields of intercropped maize and faba bean were significantly higher than those of maize and faba bean in the corresponding sole crops resulted mainly from interactions between the root systems of the two crop species. In terms of nutrient acquisition, both the structural and functional characteristics of roots have long been recognized as being important in deter-mining the capacity for plants to access and mediate the availability of essential nutrients in soil and to alleviate against those that are toxic (Darrah 1993, Hinsinger 1998). The rhizosphere is a complex environment where roots interact with physical, chemical and biological properties of soil, and is influenced by the presence and activity of root (Richardson et al. 2009). Zhang and Li (2003) indicated below-ground interactions and rhizosphere effects between intercropped crops play an important role in the advantage effect of intercropping. The objectives of this study were to (i) investigate the impact of different nitrogen fertilization levels on the advantage effect of below-ground root interaction in intercropping system under the same above-ground environmental condition, and (ii) further understand the mechanisms behind the advantage effect of interspecific below-ground root interaction by studying the effect of root interaction on the physiological characteristics, root activity and yield of intercropped soybean, and microbial quantity in the rhizosphere.

MATERIALS AND METHODS

Experimental design and management, a pot experiment was conducted outdoors at the Red Soil Experimental Station of Jiangxi Agricultural University, Nanchang City, Jiangxi province in China (28°46'N, 115°36'E) at an altitude of 22.1 ma.s.l. The annual mean temperature is 16–18°C and accumulated mean daily temperatures above 10°C are 5300–5800°C. The frost-free period is 260–280 day search year. This region is classified as having a subtropical monsoon climate. Annual precipitation is 1450–1650 mm. The soil for the pot experiment came from the red soil experimental field (0 ~ 30 cm topsoil was used) of Jiangxi Agricultural University. The experimental soil is latosolic red soil (Orthic Acrisol, FAO-UNESCO system) derived from Quaternary red earth with a sandy-loam texture (23% clay, 19% silt, 58% sand). Soil pH was 6.1, organic matter was 16.8 g/kg, total N was0.64 g/kg, total P was 0.82 g/kg, available N was 67.1 mg/kg, available P was 13.3 mg/kg, available K was 161.3 mg/kg and soil water capacity was 26.4%. The aforementioned soil properties were analyzed according to the method of Bao et al. (2000).

Plastic buckets 0.5 m high, with upper and lower diameters of 0.45 m and 0.35 m, respectively, were used. Plastic film divider was positioned in the middle of the bucket with PVC adhesive (and waterproof-glue smeared over joins to make them watertight) to produce two chambers. This produced two root separation methods: no root separation (NS, roots of crop species are allowed to completely intermingle), full root separation with plastic film (FS, both direct roots contact and the movement of any substances between the two root systems are prevented). All test buckets were divided into 4 rows and each row had 8 buckets. The distance between two buckets was always 1.0 m.

Soil well-mixed with all fertilizers (50 kg) was filled into each bucket and each chamber contained 25 kg soil. Fertilizers were applied with four nitrogen levels, i.e. N1 (0.1 g N/kg dry soil), N2 (0.3 g N/kg dry soil), N3 (0.5 g N/kg dry soil) and N4 (0.7 g N/kg dry soil), and 0.044 g P/kg dry soil and 0.083 g K/kg dry soil were used for all treatments. N was supplied as urea; P and K were applied in (NH4)2HPO4 and K2SO4 form, respectively. All fertilizers were mixed into the soil in powdered form at the beginning of the experiment. Experimental treatments consisted of four nitrogen fertilization levels (N1, N2, N3 and N4) and two root separation methods (NS and FS). This experimental design yielded 8 treatments (i.e. 4 × 2), and each treatment was replicated four times.

The selected cultivars of maize and soybean were Hengxing401 (Zea mays L., a local variety) and Zao50 (Glycine max), respectively. Maize and soybean were all sown on 25 July 2011, with 4 maize and 4 soybean seeds in each chamber of the bucket, after seedling emergence each chamber was left with 2 maize or 2 soybean plants, and all were harvested on 15 November 2011. The crop managements of each treatment were all the same. The above-ground environmental condition for each treatment was the same since the above-ground portions of each bucket had 2 maize and 2 soybean plants.

Sampling and measurements, green leaf area per plant: leaves were painted on the homogeneous and transparent paper according to their shapes and then cut down with scissors, because the texture of paper is uniform, the weight per unit area of paper was the same. Papers which were cut down can be converted into green leaf area by weighing.

Chlorophyll content: measured by using a hand-held chlorophyll meter (SPAD-502, manufactured by the Konica Minolta Company, Tokyo, Japan, measuring area: 2 mm × 3 mm), the same parts of leaves at the centre of the soybean plants were selected and analyzed at different growth stages.

The numbers of soil cultural bacteria, fungi, actinomycetes and azotobacter were counted at the maturity stage of soybean. Soil cores near the soybean roots were collected with an auger. The top 1 cm soil layer was removed and the remaining soil core (as deep as 0.2 m) was sampled. After air-drying, samples were sieved through a 1-mm sieve. Ten grams of each fresh soil sample was added to 95 mL of sterile distilled water. After homogenization for 30 min, each soil suspension was sequentially diluted and 50 μL of the resulting solutions were placed on appropriate isolation culture media. After incubation at 28°C for 4–5 days for bacteria, 3–4 days for fungi, 6–8 days for actinomycetes or 6–9 days for Azotobacter, the colony forming units (CFU) were counted. Soil bacteria, fungi, actinomycetes, and azotobacter were cultured on beef extract + peptone + agar medium, Martin medium, improved Gauss No. 1 medium, and Waksman No. 77 medium (Vieira and Nahas 2005), respectively.

Leaf photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO2 concentration were measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, USA) at 9:30–11:30 h local time at pod and maturity stages, respectively. Leaves in the upper part of soybean plant were selected for the leaf measurements.

Biological yield per plant was assessed from above-ground dry matter weight per soybean plant. For dry weight determination, samples were oven-dried at 105°C for 20 min to stop plant respiration, then oven-drying at 70°C till constant dry weight. Economic yield per plant was evaluated from grain yield per soybean plant after harvest.

Root activity was analyzed by the triphenyl tetrazolium chloride (TTC) method (Wang et al. 2006). TTC is a chemical that is reduced by dehydrogenases, mainly succinate dehydrogenase, when added to a tissue. The dehydrogenase activity is regarded as an index of the root activity. In brief, 0.5 g fresh root was immersed in 10 mL of equally mixed solution of 0.4% TTC and phosphate buffer, and kept in the dark at 37°C for 2 h. Subsequently, 2 mL of 1 mol/L H2SO4 was added to stop the reaction with the root. The root was dried with filter paper and then extracted with ethyl acetate. The red extractant was transferred into the volumetric flask to reach 10 mL by adding ethyl acetate. The absorbance of the extract at 485 nm was recorded. Root activity was expressed as TTC reduction intensity. Root activity = amount of TTC reduction (μg)/fresh root weight (g) × time (h).

Statistical analysis

The experiment was conducted as a randomized block design with four nitrogen fertilization levels and two root separation methods as the treatment variables. Analysis of variance (ANOVA) was conducted using the general linear model-univariate procedure from SPSS 17.0 software (Chicago, USA). ANOVAs were done with nitrogen fertilization level and root separation method as the main effects and including two-way interactions. All the treatment means were compared for any significant differences using the LSD's multiple range tests at a significance level of P = 0.05. Correlation analyses were also performed to evaluate the degree and significance of the correlation.

RESULTS

Green leaf area per soybean plant at maturity stage. As shown in Figure 1a, compared to the FS.

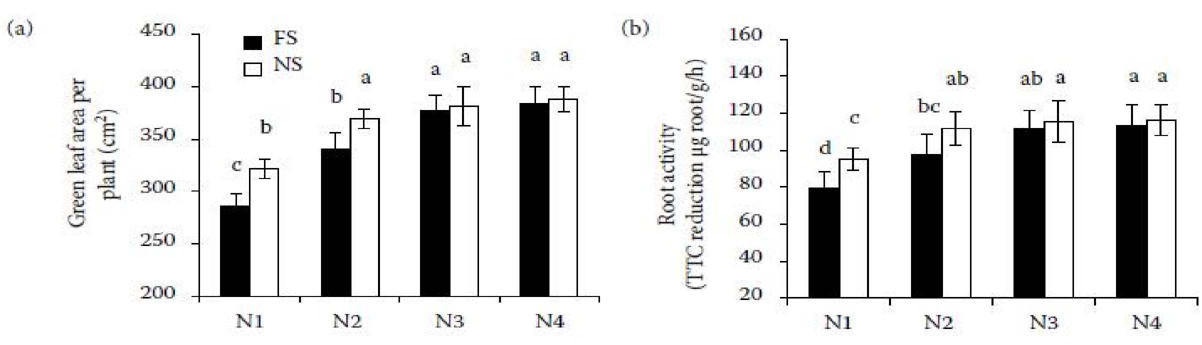

Figure 1. Effects of root separation and nitrogen fertilization (a) on green leaf area per soybean plant and (b) on the root activity of soybean at maturity stage. Values are means ± SD (n = 4).

Different letters above columns indicate a significant difference under the treatments of four nitrogen levels and two root separation methods by the LSD test (ANOVA) at the 5% level. FS – full root separation with plastic film; NS – no root separation. Nitrogen level: N1 – 0.1 g N/kg soil; N2 – 0.3 g N/kg soil; N3 – 0.5 g N/kg soil; N4 – 0.7 g N/kg soil treatment, under N1, N2, N3 and N4, NS increased green leaf area per plant by 12.8, 8.6, 1.3 and 1.1%, respectively, and the difference in green leaf area per plant between FS and NS was significant ($P < 0.05$)under N1 and N2, while insignificant under N3 and N4. Therefore, the effect of root interaction on increasing green leaf area per plant will be gradually inhibited with increasing nitrogen fertilization level. Increasing nitrogen application also enhanced the green leaf area per soybean plant at maturity stage, when N2 compared to N1, N3 compared to N2, N4 compared to N3, the green leaf area per plant was increased by 19.0, 10.8, 1.7% and 14.6, 3.3, 1.5%, respectively under FS and NS. We can draw from the above analysis data that the effect of nitrogen on increasing green leaf area per plant will be gradually decreased with increasing nitrogen fertilization level.

Table 1. Effects of nitrogen fertilization and root separation on soybean chlorophyll content at different growth stages (SPAD values).

Nitrogen fertilization	Root separation	Stage					
		initial blossoming	full blooming	initial pod	full pod	seed filling	maturity
N1	FS	23.50 ± 0.8^{d}	26.95 ± 1.4^{e}	31.68 ± 1.1^{e}	35.18 ± 0.9^{d}	38.20 ± 1.6^{c}	33.93 ± 1.6^{e}
	NS	26.28 ± 1.2^{c}	29.55 ± 0.5^{d}	34.03 ± 1.1^{d}	38.18 ± 1.0^{c}	41.58 ± 2.3^{b}	36.45 ± 0.7^{d}
N2	FS	27.93 ± 1.2^{bc}	31.88 ± 1.4^{c}	36.10 ± 0.9^{c}	39.58 ± 1.1^{c}	43.53 ± 2.0^{b}	37.43 ± 1.9^{cd}
	NS	28.85 ± 1.3^{ab}	32.40 ± 0.5^{bc}	36.70 ± 0.6^{bc}	42.32 ± 1.8^{b}	46.78 ± 0.6^{a}	39.28 ± 1.4^{bc}
N3	FS	29.25 ± 0.8^{ab}	33.48 ± 1.0^{abc}	37.48 ± 0.8^{abc}	42.78 ± 1.3^{ab}	47.68 ± 1.5^{a}	40.60 ± 1.7^{ab}
	NS	29.53 ± 0.7^{ab}	33.68 ± 1.6^{abc}	37.95 ± 0.9^{ab}	43.25 ± 1.5^{ab}	48.28 ± 2.5^{a}	41.70 ± 1.6^{a}
N4	FS	30.18 ± 1.5^{a}	33.85 ± 1.6^{ab}	37.98 ± 1.0^{ab}	44.03 ± 2.5^{ab}	48.78 ± 1.0^{a}	41.98 ± 2.3^{a}
	NS	30.43 ± 1.5^{a}	34.30 ± 1.2^{a}	38.15 ± 0.5^{a}	44.75 ± 1.1^{a}	49.18 ± 1.5^{a}	42.63 ± 1.7^{a}

Values are means ± SD (n = 4). Means followed by different letters in the same column indicate a significant difference ($P < 0.05$). FS – full root separation with plastic film; NS – no root separation. Nitrogen level: N1 – 0.1 g N/kg soil; N2 – 0.3 g N/kg soil; N3 – 0.5 g N/kg soil; N4 – 0.7 g N/kg soil

Chlorophyll content of soybean at different growth stages. As shown in Table 1, the chlorophyll content of soybean in NS treatment was higher than that in FS under the same kind of nitrogen level (N1, N2, N3 or N4). Compared to the FS treatment, under N1, NS increased chlorophyll content by 11.8, 9.6, 7.4, 8.5, 8.8 and 7.4% at initial blossoming stage, full blooming stage, initial pod stage, full pod stage, seed filling stage and maturity stage, respectively. Moreover, the difference in chlorophyll content between FS and NS at the same growth stage was significant ($P < 0.05$) under N1, while insignificant ($P > 0.05$) under N3 and N4. Increasing nitrogen application also enhanced soybean chlorophyll content, and the differences in chlorophyll content at the same growth stage between N1 and N2 were the most significant, while between

N3 and N4 they were all insignificant ($P > 0.05$). Therefore, the effect of root interaction and nitrogen on the increase of chlorophyll content will be gradually inhibited or decreased with increasing nitrogen fertilization level.

Photosynthetic characteristics of soybean. Under the same kind of nitrogen level (N1, N2, N3 or N4), photosynthetic rate, stomatal conductance and transpiration rate in NS treatment were all higher than those in FS, but intercellular CO2 concentration was lower than that in FS (Table 2). Compared to the FS treatment, under N1, NS increased photosynthetic rate, stomatal conductance and transpiration rate by 36.1, 35.7, 30.7% and 32.4, 55.6, 27.6%, while reduced intercellular CO2 concentration by 10.4% and 6.9%, respectively at pod and maturity stages. Moreover, the differences in photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO2 concentration at the same growth stage between NS and FS were significant ($P < 0.05$) under N1, while insignificant ($P > 0.05$) under N3 and N4. Increasing nitrogen application also improved the photosynthetic characteristics of soybean, and the differences in photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO2 concentration at the same growth stage between N1 and N2 were the most significant, while between N3 and N4 were the most insignificant ($P > 0.05$). Therefore, the effect of root interaction and nitrogen on improving the photosynthetic characteristics of soybean will be gradually inhibited or decreased with increasing nitrogen fertilization level.

Table 2. Effects of nitrogen fertilization and root separation on photosynthetic characteristics of soybean at pod and maturity stages

Nitrogen fertilization	Root separation	Photosynthetic rate (μmol/m^2/s)	Stomatal conductance (mol/m^2/s)	Transpiration rate (mmol/m^2/s)	Intercellular CO_2 concentration (μmol/mol)
Pod stage					
N1	FS	9.50 ± 1.33^{e}	0.14 ± 0.03^{d}	1.66 ± 0.18^{d}	330.45 ± 19.63^{a}
	NS	12.93 ± 2.41^{d}	0.19 ± 0.04^{c}	2.17 ± 0.21^{c}	296.08 ± 23.93^{b}
N2	FS	15.63 ± 1.72^{c}	0.26 ± 0.03^{b}	2.30 ± 0.25^{c}	266.58 ± 21.09^{c}
	NS	16.40 ± 2.05^{bc}	0.29 ± 0.03^{b}	2.84 ± 0.20^{b}	256.30 ± 16.02^{cd}
N3	FS	17.83 ± 2.12^{abc}	0.35 ± 0.03^{a}	3.11 ± 0.48^{ab}	242.73 ± 13.15^{cde}
	NS	18.30 ± 2.16^{ab}	0.37 ± 0.04^{a}	3.25 ± 0.33^{ab}	231.53 ± 16.36^{def}
N4	FS	19.20 ± 2.54^{a}	0.38 ± 0.05^{a}	3.27 ± 0.34^{ab}	226.33 ± 14.02^{ef}
	NS	19.73 ± 2.31^{a}	0.39 ± 0.05^{a}	3.32 ± 0.35^{a}	212.83 ± 12.91^{f}
Maturity stage					
N1	FS	2.38 ± 0.28^{e}	0.09 ± 0.02^{f}	0.98 ± 0.09^{f}	391.48 ± 10.39^{a}
	NS	3.15 ± 0.26^{d}	0.14 ± 0.02^{e}	1.25 ± 0.05^{e}	364.48 ± 18.10^{b}
N2	FS	3.23 ± 0.39^{d}	0.18 ± 0.01^{d}	1.29 ± 0.12^{de}	330.45 ± 16.92^{c}
	NS	3.93 ± 0.50^{c}	0.20 ± 0.02^{cd}	1.42 ± 0.07^{cd}	315.20 ± 13.55^{cd}
N3	FS	3.85 ± 0.37^{c}	0.21 ± 0.02^{bc}	1.51 ± 0.05^{bc}	316.45 ± 12.42^{cd}
	NS	3.98 ± 0.22^{bc}	0.24 ± 0.03^{abc}	1.65 ± 0.18^{ab}	299.70 ± 12.63^{de}
N4	FS	4.43 ± 0.26^{ab}	0.25 ± 0.02^{ab}	1.69 ± 0.11^{a}	285.60 ± 13.02^{ef}
	NS	4.63 ± 0.31^{a}	0.26 ± 0.04^{a}	1.73 ± 0.14^{a}	265.35 ± 15.96^{f}

Values are means ± SD ($n = 4$). Means followed by different letters in the same column indicate a significant difference ($P < 0.05$). FS – full root separation with plastic film; NS – no root separation. Nitrogen level: N1 – 0.1 g N/kg soil; N2 – 0.3 g N/kg soil; N3 – 0.5 g N/kg soil; N4 – 0.7 g N/kg soil

Microbial quantity in rhizosphere, under the same kind of nitrogen level (N1, N2, N3 or N4), the number of culturable bacteria, fungi, actinomycetes and Azotobacteria in soybean rhizosphere in NS treatment was higher than that in FS (Table 3).

Compared to the FS treatment, under N1, NS increased the number of bacteria, fungi, actinomycetes and Azotobacteria by 44.3, 92.9, 105.0 and 93.3%, respectively. Moreover, the difference in the number of bacteria, fungi, actinomycetes and Azotobacteria between NS and FS was significant ($P < 0.05$) under N1, while insignificant ($P > 0.05$) under N3 and N4. Increasing nitrogen application also increased the number of bacteria, fungi and actinomycetes in soil, and the differences in the number of bacteria, fungi and actinomycetes between N1 and N2 treatments were the most significant, while between N3 and N4 were the most insignificant ($P > 0.05$). In addition, the number of Azotobacteria in soil presented a change trend of first increased and then decreased with increas¬ing levels of nitrogen. Therefore, root interaction and nitrogen fertilization can increase microbial quantity in soybean rhizosphere, but their effects will be gradually inhibited or decreased with increasing nitrogen fertilization level.

The root activity of soybean at maturity stage. As shown in Figure 1b, compared to FS treat¬ment, NS increased root activity by 20.1, 14.3, 3.1 and 2.6% respectively under N1, N2, N3 and N4. Moreover, the difference in root activity between FS and NS was significant ($P < 0.05$) under N1, while insignificant under N2, N3 and N4. Therefore, the effect of root interaction on increasing the root activity of soybean will be gradually inhibited with increasing nitrogen fertilization level. Increasing nitrogen application also enhanced the root activity of soybean at maturity stage, when N2 compared to N1, N3 compared to N2, N4 compared to N3, root activity were increased by 23.3, 14.5, 1.1% and 17.4, 3.2, 0.6%, respectively under FS and NS. We can conclude from the above analysis data that the effect of nitrogen on increasing root activity will be gradually decreased with increasing nitrogen fertilization level.

Table 3. Effects of nitrogen fertilization and root separation on microbial quantity in soybean rhizosphere at maturity stage.

Nitrogen fertilization	Root separation	Bacteria (× 10^6 CFU/g dry soil)	Fungi (× 10^4 CFU/g dry soil)	Actinomycetes (× 10^5 CFU/g dry soil)	Azotobacteria (× 10^4 CFU/g dry soil)	Azotobacteria/ bacteria (%)
N1	FS	17.50 ± 5.32^{d}	3.50 ± 1.29^{e}	5.00 ± 2.16^{d}	3.75 ± 1.71^{c}	0.23 ± 0.13^{ab}
	NS	25.25 ± 4.50^{c}	6.75 ± 2.22^{d}	10.25 ± 2.99^{c}	7.25 ± 1.71^{a}	0.30 ± 0.10^{a}
N2	FS	31.75 ± 3.77^{b}	8.25 ± 2.50^{cd}	13.25 ± 2.22^{bc}	6.50 ± 2.08^{ab}	0.20 ± 0.05^{abc}
	NS	34.50 ± 4.73^{ab}	10.75 ± 2.63^{abc}	18.75 ± 3.10^{a}	8.00 ± 1.83^{a}	0.23 ± 0.06^{ab}
N3	FS	32.25 ± 3.77^{b}	9.25 ± 2.63^{bcd}	17.25 ± 3.30^{ab}	4.50 ± 1.29^{bc}	0.14 ± 0.04^{bc}
	NS	37.00 ± 4.69^{ab}	11.75 ± 1.71^{ab}	19.25 ± 3.50^{a}	6.50 ± 1.29^{ab}	0.18 ± 0.05^{bc}
N4	FS	36.00 ± 3.74^{ab}	11.75 ± 1.71^{ab}	18.75 ± 2.99^{a}	3.75 ± 2.22^{c}	0.10 ± 0.06^{c}
	NS	38.75 ± 2.99^{a}	12.50 ± 1.29^{a}	20.75 ± 3.10^{a}	4.25 ± 1.71^{bc}	0.11 ± 0.05^{c}

Values are means ± SD ($n = 4$). Means followed by different letters in the same column indicate a significant difference ($P < 0.05$). FS – full root separation with plastic film; NS – no root separation. Nitrogen level: N1 – 0.1 g N/kg soil; N2 – 0.3 g N/kg soil; N3 – 0.5 g N/kg soil; N4 – 0.7 g N/kg soil

Soybean yield and its components. Under the same kind of nitrogen level (N1, N2, N3 or N4), plant height, main stem node number, pods per plant, grains per plant, economic and biological yields per soybean plant in NS treatment were all higher than those in FS, while bottom pod height was lower than that in FS (Table 4). Compared to FS treatment, under N1, NS increased plant height, grains per plant, economic and biological yields per plant by 12.0, 19.7, 18.1 and 14.2%, respectively. Moreover, the differences in plant height, grains per plant, economic and biological yields per plant between FS and NS were significant ($P < 0.05$) under N1, while insignificant under N2, N3 and N4. Increasing nitrogen application also enhanced soybean yield and improved its components, and the differences in plant height, bottom pod height, main stem node number, pods per plant, grains per plant, economic and biological yields per plant between N1 and N2 were the most significant($P < 0.05$), while between N3 and N4 were the most insignificant ($P > 0.05$). Therefore, the effects of root interaction and nitrogen on increasing soy¬bean yield and improving yield components will be gradually inhibited or decreased with increasing nitrogen fertilization level.

Table 4. Effects of root separation and nitrogen fertilization on soybean yield and its components.

Nitrogen fertilization	Root separation	Plant height (cm)	Bottom pod height (cm)	Main stem node number	Pods per plant (individual)	Grains per plant (individual)	Economic yield per plant (g)	Biological yield per plant (g)
N1	FS	45.1 ± 3.7^{e}	13.9 ± 0.5^{a}	7.9 ± 0.9^{d}	22.8 ± 3.1^{d}	45.8 ± 3.8^{f}	7.2 ± 0.6^{e}	36.5 ± 3.1^{d}
	NS	50.5 ± 5.0^{d}	13.1 ± 0.8^{ab}	8.1 ± 0.7^{d}	25.0 ± 2.6^{d}	54.8 ± 4.0^{e}	8.5 ± 0.5^{d}	41.7 ± 3.1^{c}
N2	FS	57.6 ± 3.7^{c}	12.0 ± 0.6^{cd}	8.7 ± 0.8^{cd}	32.8 ± 4.9^{c}	60.3 ± 2.8^{d}	10.1 ± 0.5^{c}	45.5 ± 2.9^{bc}
	NS	59.7 ± 3.1^{bc}	11.7 ± 0.7^{cd}	8.9 ± 1.6^{bcd}	34.8 ± 4.3^{bc}	64.8 ± 4.6^{cd}	10.8 ± 0.7^{c}	49.1 ± 2.4^{ab}
N3	FS	63.9 ± 2.6^{ab}	11.3 ± 0.4^{cd}	9.7 ± 0.8^{abc}	36.5 ± 3.7^{abc}	68.8 ± 2.5^{bc}	12.2 ± 0.8^{b}	50.0 ± 2.5^{ab}
	NS	66.5 ± 2.6^{a}	11.2 ± 0.9^{d}	10.0 ± 0.8^{ab}	37.8 ± 4.1^{ab}	70.8 ± 3.6^{ab}	12.8 ± 0.3^{ab}	50.6 ± 3.8^{a}
N4	FS	67.3 ± 2.2^{a}	11.9 ± 0.7^{bc}	10.3 ± 1.1^{a}	38.5 ± 3.7^{ab}	73.3 ± 4.5^{ab}	13.0 ± 0.3^{ab}	51.4 ± 2.8^{a}
	NS	68.1 ± 3.4^{a}	11.4 ± 1.1^{cd}	10.4 ± 1.7^{a}	40.8 ± 3.4^{a}	74.3 ± 3.3^{a}	13.1 ± 0.9^{a}	52.4 ± 3.7^{a}

Values are means ± SD ($n = 4$). Means followed by different letters in the same column indicate a significant difference ($P < 0.05$). FS – full root separation with plastic film; NS – no root separation. Nitrogen level: N1 – 0.1 g N/kg soil; N2 – 0.3 g N/kg soil; N3 – 0.5 g N/kg soil; N4 – 0.7 g N/kg soil

Relationship between root activity and green leaf area per plant, chlorophyll content, photosynthetic rate, and economic yield per plant.

Figure 2 shows that the root activity of soybean was significantly ($P < 0.05$) positively correlated with green leaf area per plant, chlorophyll content, photosynthetic rate and economic yield per plant with the shape of a straight line, and the coefficient of determination (R2) were all above 0.90, indicating that the increase of root activity can help to enhance green leaf area per plant, chlorophyll content, photosynthetic rate and economic yield per soybean plant.

DISCUSSION

If N was applied after fruit set, less was taken up and most of that was stored in the roots. While early applications were more likely to move into new vegetation and fruit, high rates early in the season were associated with excessive vegetative growth. As a result of this research, a moderate N rate split into 4-5 applications was recommended for Oregon, with early applications limited unless tissue tests from the previous season showed N deficiency.

Interspecific facilitation (or positive interaction) is a phenomenon when one plant species enhances the survival, growth, or fitness of another, and was demonstrated in many natural plant communities (Callaway and Pugnaire 1999). In this study, compared to FS treatment, under N1, N2, N3 and N4, NS increased green leaf area per plant by 12.8, 8.6, 1.3 and 1.1%, respectively; indicating that root interaction can help to increase the green leaf area per soybean plant at maturity stage, but its effect will be gradually inhibited with increasing nitrogen fertilization level. Zuo et al. (2000) indicated that the rhizosphere interactions between peanut

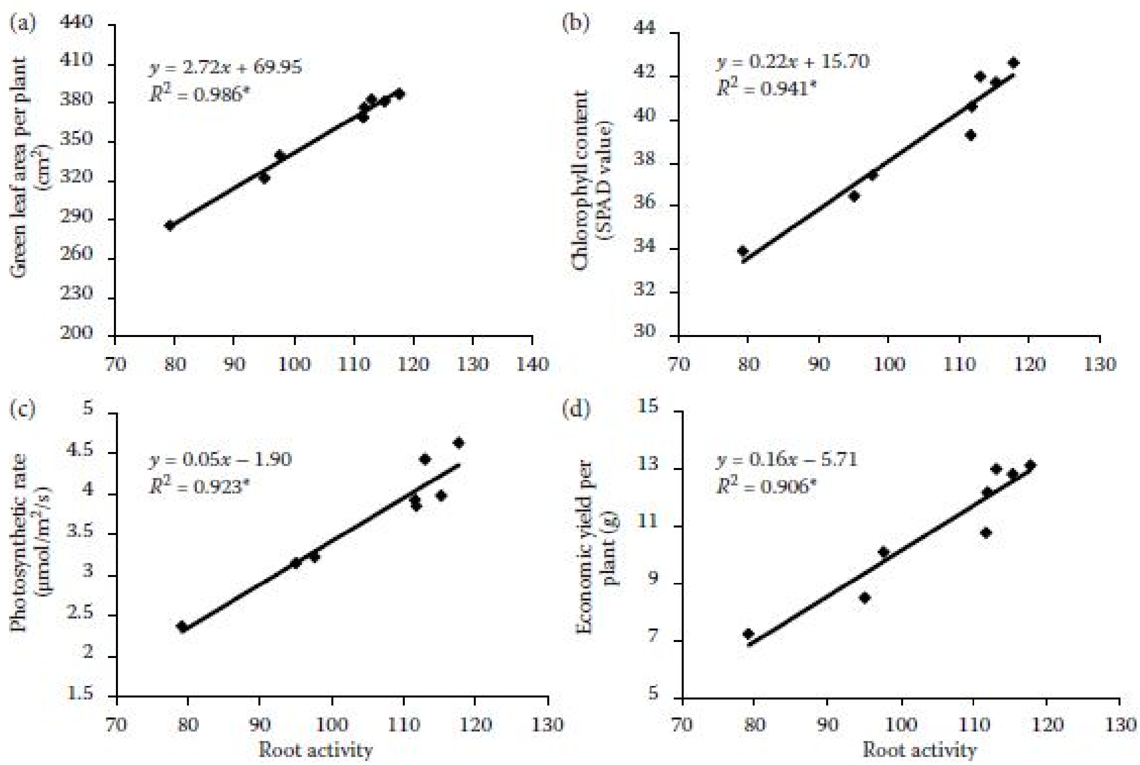

Figure 2. Relationship between root activity and green leaf area per plant (a), chlorophyll content (b), photosynthetic rate (c) and economic yield per plant (d). *P < 0.01.

Plant Soil Environ. Vol. 59, 2013, No. 2: 80–88 and maize improved the Fe nutrition of peanut and enhanced chlorophyll content of peanut. In this study, we not only found that root interaction increased soybean chlorophyll content at different growth stages, but also found that the effects of root interaction and nitrogen were gradually inhibited or decreased with increasing nitrogen fertilization level.

The improvement of photosynthetic characteristics has a significant impact on crop yield, growth and development (Anten 2005).

In this study, we found that root interaction and increasing nitrogen application could improve the photosynthetic characteristics of soybean, but their effects were gradually inhibited or decreased with increasing nitrogen fertilization level.

Soil microbial quantity can have a strong influence on plant growth and productivity by altering root growth via hormone production or nutrient availability through mineralization (Van der Heijden et al. 2008, Richardson et al. 2009, Fan et al. 2011). In this study, we found that root interaction could increase the root activity of soybean and microbial quantity in the rhizosphere, but its effect was gradually inhibited with increasing nitrogen fertilization level. The reason why inter specific root interaction can increase soil microbial quantity mainly due to plants in intercropping system exert species-specific effects on the rhizosphere microbial community and quantity as a result of differences in amount and composition of root exudates (Richardson et al. 2009, Dennis et al. 2010). The increase of the root activity of soybean mainly due to root interaction in intercropping system can improve rhizosphere soil microbial environment (Hinsinger et al. 2011), physical and chemical properties (Song et al. 2007, Richardson et al. 2009), and the growth and development status of crop (Hauggaard-Nielsen and Jensen 2005, Zhang et al. 2010). This study also found that increasing nitrogen application could increase microbial quantity in rhizosphere and enhance the root activity of soybean, but its effect was gradually decreased with increasing nitrogen fertilization level. In addition, the number of Azotobacteria in soil had a change trend of first increased and then decreased with increasing nitrogen fertilization level, indicating that high nitrogen level had a great inhibitory effect on the number of Azotobacteria.

Li et al. (2011) indicated that the yield from intercropping was higher than that expected from the yields of component species under sole cropping. Losak (2007) found that increasing levels of nitrogen increased soybean yield, improved its crude protein content and yield components. In this study, we found that root interaction and increasing nitrogen application could increase soybean yield and improve its components, but their effects were gradually inhibited or decreased with increasing nitrogen fertilization level. Correlation analysis revealed that the root activity of soybean was significantly ($P < 0.05$) positively correlated with green leaf area per plant, chlorophyll content, photosynthetic rate and economic yield per plant. The above portion of this paper has proved that root interaction could increase the root activity of soybean, so this correlation analysis reconfirmed from another side that root interaction could improve some physiological indexes and increase soybean yield by enhancing root activity. Because the advantage effects of root interaction and increasing nitrogen application were gradually inhibited or decreased with increasing nitrogen fertilization level, in agricultural production practices, we should pay attention to taking reasonable intercropping patterns to achieve high and stable crop yields by fully playing the advantage effect of root interaction with low nitrogen fertilization level, so as to achieve the coordination and unification of economic and ecological benefits.

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