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RESEARCH

Differences in maize physiological characteristics, nitrogen accumulation, and yield under different cropping patterns and nitrogen levels

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Intercropping and N fertilization play an important role in increasing crop yield. In order to further understand the advantage mechanism of intercropping and the effect of increasing N application on the advantage effect of intercropped crop, a field experiment was conducted to investigate the effects of different cropping patterns (i.e. M, maize monoculture; I1, maize-cotton intercrop; I2, maize-soybean intercrop) and N fertilization levels (N1, 100 kg ha^{-1}; N2, 200 kg ha^{-1}; N3, 300 kg ha^{-1}; N4, 400 kg ha^{-1}) on maize (*Zea mays* L.) Results showed that intercropping and increasing N application could enhance green leaf area per maize plant and chlorophyll content, and differences in green leaf area per plant and chlorophyll content between intercropping and monoculture under N1 were significant. Intercropping and increasing N application could improve maize photosynthetic characters, but their effects would be decreased with increasing N fertilization level. Root bleeding sap rate and root DM of maize were also obviously affected by intercropping and N fertilization, and the differences in root bleeding sap rate and root DM between I2 and M under N1 and N2 were significant. Compared to M, under N1, N2, N3, and N4, I2 increased grain N content by 12.8%, 6.3%, 2.7%, 1.5%, respectively. Intercropping and increasing N application could increase maize yield, and the difference in yield between I2 and M under N1 was significant. All the findings suggest that intercropping and increasing N application can improve maize physiological characters and increase maize root DM, N accumulation and yield, but their effects will be decreased with increasing N fertilization level.

Key words: Chlorophyll content, nitrogen content, photosynthetic characters, root dry mass yield, *Zea mays*.

INTRODUCTION

Intercropping is a cropping pattern with a 1000-yr-old history in Chinese agriculture, and it is still widespread in modern Chinese agriculture. Intercropping has the potential to achieve higher grain yields than monoculture systems by mainly improving the efficient use of water, light, nutrients, and other resources (Li et al., 2011). Enhanced productivity of intercropping compared with monoculture can be explained by two major processes that result in improved resource use: complementarity and facilitation (Fridley, 2001). Complementarity can be defined as a decrease in interspecific competition and competitive exclusion through resource partitioning between intercropped species (Hinsinger et al., 2011). For example, species can use a given resource differently based on time, space, and characteristics. Facilitation occurs when one species enhances the growth or survival of another (Callaway, 1995). This can occur through (1) direct positive mechanisms, such as favorable alteration of temperature, light, soil moisture, and nutrients and (2) indirect mechanisms, such as beneficial changes in soil mycorrhizal or microbial communities.

Nowadays, most studies of intercropping have been focused on low-input and low-output systems (Kavamahanga et al., 1995; Hauggaard-Nielsen and Jensen, 2001) to develop organic farming systems. In contrast, intercropping in China has developed using intensive farming systems with high inputs and high outputs, typically cereal/cereal systems. However, the advantageous effect of an intercrop would change due to high fertilizer inputs because soil fertility plays an important role in interspecific interactions between intercrops. For example, some agronomists have indicated that higher nutrient availability aggravates interspecific competition because larger individuals per se occupy a higher development space, intercept more light, and assimilate more nutrient resources and water (Keddy, 1989; Schippers et al., 1999), However, Li et al. (2011) indicated that increasing N application rates alleviated the competitive intensity of intercrops in cereal/cereal intercropping.

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This study therefore researched the difference in maize (*Zea mays* L.) physiological characteristics, N accumulation, and yield between monoculture and intercropping under different N fertilization levels. The aims were to investigate the effect of increasing N applications on the advantageous effect of intercrops, and clarify the change in the trend of the effect of N fertilizer by enhancing the N fertilization level. We hypothesized that the advantageous effects of intercropping were only significant for the low N fertilization level (N1), and the effects of increasing N applications would decrease by increasing the N fertilization level.

MATERIALS AND METHODS

Experimental design and management

The field experiment was conducted in 2012 and 2013 at the Red Soil Experimental Station of Jiangxi Agricultural University, Nanchang City (28°46' N, 115°36' E; 22 m a.s.l.), Jiangxi province in China. Annual mean temperature is 16-18 °C and accumulated temperatures above 10 °C were 5300-5800 °C. The frost-free period is 260-280 d per year. The region is classified as a subtropical monsoon climate. Annual precipitation is 1450-1650 mm. The soil used in the experiment is latosolic red soil (Orthic Acrisol, FAO-UNESCO system) derived from Quaternary red earth with a sandy-loam texture (58% sand, 19% silt, 23% clay). Soil properties were pH 6.0, 17.4 g kg^{-1} organic matter , 0.68 g kg^{-1} total N , 0.76 g kg^{-1} total P , 64.2 g kg^{-1} available N , 12.9 g kg^{-1} available P , 151.6 g kg^{-1} available K, and 25.7% soil water capacity. These were analyzed according to the method by Bao et al. (2000).

The experiment was conducted in a randomized block design with four N fertilization levels (N1, N2, N3, and N4) and three cropping patterns (M, I1, and I2) as the treatment variables. This experimental plan generated 12 treatments (i.e., 4 × 3) and each treatment was replicated three times. The four N levels were N1 (100 kg ha^{-1}), N2 (200 kg ha^{-1}), N3 (300 kg ha^{-1}), and N4 (400 kg ha^{-1}); and the three cropping patterns were M (maize monoculture), I1 (maize-cotton intercrop), and I2 (maize-soybean intercrop). All plots were given a basal application of 300 kg P ha^{-1} and 200 kg K ha^{-1}. Nitrogen was supplied as urea and $(NH_4)_2HPO_4$, and P and K were applied as $(NH_4)_2HPO_4$ and K_2SO_4, respectively. All fertilizers were evenly broadcasted and incorporated into the top 20 cm of the soil prior to sowing.

Each plot was 33.0 m^2 (5.5 m × 6 m) with 50 cm row spacing between neighboring plots. The selected maize, soybean (*Glycine max* [L.] Merr.), and cotton (*Gossypium hirsutum* L.) cultivars were 'Hengxing401' (local variety), 'Zao50', and 'Ganmian1', respectively. Maize, soybean, and cotton were all sown on 2 April 2012 and 2013, and maize was harvested on 18 July each year. Each intercropping plot consisted of two maize strips and three soybean or cotton strips. Each soybean and cotton strip had five planted rows, and each maize strip had two planted rows. There were 19 plants per row for maize, and each intercropping plot had 76 maize plants. Row and plant spacing for maize was 40 and 30 cm, respectively, and row and plant spacing for soybean and cotton were both 30 cm, while row spacing between maize and adjacent soybean (or cotton) was 40 cm. In addition, row and plant spacing in monoculture maize was the same as in intercropping.

Sampling and measurements

Green leaf area per plant. Leaves were painted on homogeneous and transparent paper according to their shapes and then cut down with scissors because the paper texture is uniform and the weight per unit area of paper was the same. Papers which had been cut down could be converted into green leaf area by weighing. Five maize plants in each plot were selected for measurements at the maturity stage.

Chlorophyll content. It was measured with a hand-held chlorophyll meter (SPAD-502, Konica Minolta Company, Tokyo, Japan), the same leaf parts in the middle of the maize plant were selected and measured at the bell-mouthed, silking, filling, and maturity stage, respectively.

Leaf photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration were measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, Nebraska, USA) at 10:00 to 11:30 h local time at the filling stage. Maize ear leaves were selected for leaf measurements and each leaf was measured in three points.

Leaf and grain N content was analyzed by the Kjeldahl N determination method (Wang et al., 2006); N accumulation (g plant^{-1}) = N content of samples (leaves and grain) × Total biomass of samples per plant.

Root dry mass. The approximate depth of 0 to 0.5 m and width of 0.3 m of maize roots in soil were taken at the maturity stage; five maize plants were selected from each plot. Root samples (washed clean with water) were oven-dried at 105 °C for 20 min to stop respiration, oven-dried at 70 °C for 48 h, and weighed immediately after being removed from the oven.

Root bleeding sap rate. Five maize stems were selected from each plot and cut at a distance of 5 cm from the soil surface. Dry cotton (previously weighed) was placed at the cut end, covered with a vinyl film fastened with a rubber band to collect the bleeding sap for 12 h (from 18:00 h on 20 June to 06:00 h on 21 June), and then weighed to calculate the bleeding rate (Morita et al., 2000; Song and Li, 2003).

Biomass per plant. This was above-ground DM weight per maize plant. Five maize plants were selected from each plot to determine dry weight; samples were oven-dried at 105 °C for 30 min to stop plant respiration and then oven-dried at 70 °C until constant dry weight.

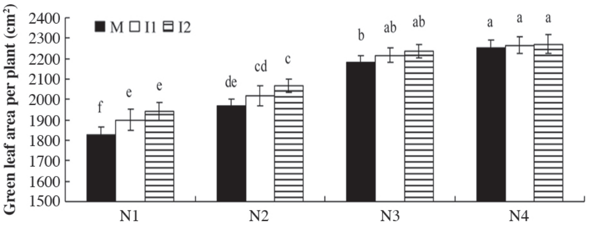
Statistical analysis

ANOVA was performed by the general linear model-univariate procedure from SPSS17.0 software (IBM, Armonk, New York, USA). ANOVAs were performed with the N level and cropping pattern as the main effects as well as their interaction. All treatment means were compared for any significant differences by the LSD multiple range tests at the significant level of $P < 0.05$ with the SPSS17.0 software package for Windows.

RESULTS

Green leaf area per plant and chlorophyll content at different growth stages in maize

Nitrogen fertilization and the cropping pattern had a significant effect on green leaf area per plant, but their interaction was not significant (Figure 1). When compared with the M treatment, I1 and I2 for N1, N2, N3, and N4 increased green leaf area per plant by 3.8%, 2.5%, 1.7%, and 0.5%, and 6.1%, 5.0%, 2.7%, and 0.7%, respectively; the difference between intercropping and monoculture for N1 was significant ($P < 0.05$). Increasing N applications can also enhance green leaf area per plant in maize under M, I1, and I2; when N2 was compared with N1 and N4 was compared with N3, green leaf area per plant increased by 7.6%, 6.2%, and 6.5% and 3.4%, 2.3%, and 1.4%, respectively. Therefore, data from the above analysis can be used to infer that intercropping and increasing N applications can increase green leaf area per plant in maize, but that their effects will gradually decrease with increasing N fertilization levels.



Values are means ± standard deviation (n = 3). Different letters above columns indicate differences for treatments of four N levels and three cropping patterns according to LSD tests (ANOVA) at the 5% level. (N fertilization $P < 0.01$; Cropping pattern $P < 0.01$; N fertilization × Cropping pattern $P = 0.5527$).
M: maize monoculture; I1: maize-cotton intercrop; I2: maize-soybean intercrop; N1: 100 kg ha^{-1}; N2: 200 kg ha^{-1}; N3: 300 kg ha^{-1}; N4: 400 kg ha^{-1}.

Figure 1. Effects of intercropping and N fertilization on green leaf area per plant in maize at the maturity stage.

Nitrogen fertilization and the cropping pattern had a significant effect on chlorophyll content but with no interaction. Within the same N level (N1, N2, N3, or N4), chlorophyll content of intercropped maize at different growth stages was higher than for monoculture (Table 1). When compared with the M treatment, I1 and I2 for N1, N2, N3, and N4 increased chlorophyll content by 7.1%, 4.9%, 4.5%, and 9.4%, and 10.7%, 7.5%, 5.6%, and 10.9%, respectively, at the bell-mouthed, silking, filling, and maturity stage, respectively. The difference in chlorophyll content between intercropping and monoculture for N1 was significant ($P < 0.05$), while it was not significant for N3 and N4. Increasing N applications can also enhance the chlorophyll content of maize; the difference in chlorophyll content of the same cropping pattern (M, I1, or I2) between N1 and N2 was significant, while it was not significant between N3 and N4 ($P > 0.05$). Therefore, the effect of intercropping and N fertilizer on increasing chlorophyll content of maize will gradually decrease with increasing N fertilization levels.

Photosynthetic characteristics

Nitrogen fertilization and the cropping pattern had a significant effect on maize photosynthetic characteristics, but their interaction was only different for the photosynthetic rate (Table 2). When compared with monoculture,

Table 1. Effects of intercropping and N fertilization on maize chlorophyll content at different growth stages (SPAD value).

N fertilization	Cropping patterns	Bell-mouthed stage	Silking stage	Filling stage	Maturity stage
N1	M	35.91 ± 1.34h	48.89 ± 0.77f	54.55 ± 1.46e	36.79 ± 1.22e
	I1	38.46 ± 0.56g	51.31 ± 2.02e	56.99 ± 0.93d	40.25 ± 1.19d
	I2	39.74 ± 0.94fg	52.55 ± 1.94de	57.58 ± 1.52cd	40.79 ± 1.03cd
N2	M	40.91 ± 0.85ef	53.74 ± 0.94d	59.50 ± 1.04bc	41.29 ± 1.13bcd
	I1	42.03 ± 1.00de	54.56 ± 1.26cd	61.38 ± 0.84ab	43.12 ± 0.96abc
	I2	42.74 ± 1.45cde	56.64 ± 0.81bc	62.13 ± 1.43a	43.66 ± 1.06ab
N3	M	43.93 ± 1.18bcd	56.81 ± 2.22bc	61.61 ± 1.69ab	43.72 ± 2.68ab
	I1	44.61 ± 1.16abc	57.77 ± 1.42ab	62.52 ± 1.94a	44.68 ± 1.48a
	I2	44.80 ± 1.48abc	58.18 ± 1.07ab	62.92 ± 1.50a	44.91 ± 2.72a
N4	M	45.89 ± 0.81ab	58.86 ± 0.68ab	63.04 ± 1.34a	45.16 ± 1.23a
	I1	46.25 ± 1.76a	59.77 ± 1.23a	63.32 ± 1.22a	45.58 ± 1.33a
	I2	46.35 ± 1.52a	60.09 ± 1.65a	63.55 ± 2.21a	45.72 ± 2.07a
Significance LSD multiple range tests (P values)					
N fertilization		0.0001	0.0001	0.0001	0.0001
Cropping pattern		0.0068	0.0024	0.0118	0.0158
N fertilization × Cropping pattern		0.3664	0.6700	0.7476	0.6320

Values are means ± standard deviation (n = 3). Means followed by different letters in the same column indicate differences according to LSD multiple range tests ($P < 0.05$).
M: maize monoculture; I1: maize-cotton intercrop; I2: maize-soybean intercrop; N1: 100 kg ha^{-1}; N2: 200 kg ha^{-1}; N3: 300 kg ha^{-1}; N4: 400 kg ha^{-1}.

Table 2. Effects of intercropping and N fertilization on maize photosynthetic characteristics at the filling stage.

N fertilization	Cropping patterns	Photosynthetic rate	Stomatal conductance	Transpiration rate	Intercellular CO_2 concentration
		μmol m^{-2} s^{-1}	mol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	μmol mol^{-1}
N1	M	23.27 ± 2.75g	0.22 ± 0.03g	3.49 ± 0.28g	135.20 ± 6.31a
	I1	29.87 ± 2.11f	0.28 ± 0.03f	4.52 ± 0.39f	105.83 ± 9.19bc
	I2	29.27 ± 0.76f	0.30 ± 0.03ef	4.71 ± 0.50f	108.27 ± 8.90bc
N2	M	33.40 ± 0.96e	0.29 ± 0.02f	4.43 ± 0.43f	118.77 ± 11.08b
	I1	38.47 ± 1.21d	0.34 ± 0.02cde	5.53 ± 0.28de	100.73 ± 7.55cd
	I2	39.67 ± 1.60cd	0.36 ± 0.03bcd	5.78 ± 0.20bcd	95.70 ± 8.61cde
N3	M	37.43 ± 1.20bcd	0.32 ± 0.03def	5.24 ± 0.23e	106.77 ± 13.18bc
	I1	40.27 ± 1.26bc	0.38 ± 0.03abc	6.12 ± 0.57abc	90.53 ± 5.50de
	I2	41.97 ± 1.60ab	0.40 ± 0.03ab	6.27 ± 0.35ab	88.67 ± 7.31de
N4	M	41.77 ± 0.96ab	0.39 ± 0.02ab	5.61 ± 0.35cde	97.17 ± 9.36cde
	I1	42.07 ± 2.30ab	0.42 ± 0.04a	6.47 ± 0.34a	85.73 ± 10.92e
	I2	43.53 ± 0.60a	0.43 ± 0.05a	6.60 ± 0.35a	86.47 ± 8.77de
Significance LSD multiple range tests (P values)					
N fertilization		0.0001	0.0001	0.0001	0.0001
Cropping pattern		0.0001	0.0001	0.0001	0.0001
N fertilization × Cropping pattern		0.0368	0.7615	0.9517	0.5933

Values are means ± standard deviation (n = 3). Means followed by different letters in the same column indicate differences according to LSD multiple range tests ($P < 0.05$).

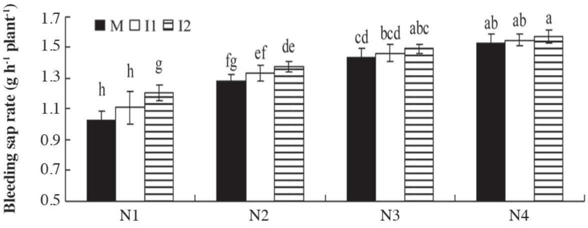
M: maize monoculture; I1: maize-cotton intercrop; I2: maize-soybean intercrop; N1: 100 kg ha^{-1}; N2: 200 kg ha^{-1}; N3: 300 kg ha^{-1}; N4: 400 kg ha^{-1}.

intercropping can increase leaf photosynthetic rate, stomatal conductance, and transpiration rate, while it can decrease intercellular CO_2 concentration. Moreover, the difference between intercropping and monoculture for N1 and N2 was significant ($P < 0.05$), while it was not significant for N4 (with the exception of transpiration rate). Increasing N applications can also improve maize photosynthetic characteristics; the differences in photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration of the same cropping pattern (M, I1, or I2) between N1 and N2 were mostly significant, while they were mostly not significant ($P > 0.05$) between N3 and N2, and N4 and N3. Therefore, the effect of intercropping and N fertilizer to improve maize photosynthetic characteristics will gradually decrease with increasing N fertilization levels.

Root bleeding sap rate and root dry mass per plant

When compared with the M treatment, I1 and I2 for N1, N2, N3, and N4 increased root bleeding sap rate by 7.8%, 3.9%, 1.4%, and 1.3%, and 16.5%, 7.0%, 3.5%, and 2.6%, respectively; the difference between I2 and M for N1 and N2 was significant ($P < 0.05$), while it was not significant for N3 and N4 (Figure 2). In addition, for M, I1, and I2, N2 increased root bleeding sap rate by 25.2%, 20.7%, and 15.0% as compared with N1; N3 increased by 11.6%, 9.0%, and 8.0% as compared with N2; and N4 increased by 6.3%, 6.2%, and 5.4% as compared with N3. Intercropping and increasing N applications can increase the root bleeding sap rate of maize, but their effects will gradually decrease with increasing N fertilization levels.

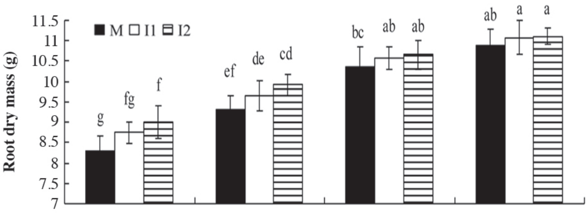
Nitrogen fertilization and the cropping pattern had an effect on root DM per plant ($P < 0.005$, Figure 3). When compared with the M treatment, I1 and I2 for N1, N2, N3, and N4 increased root DM by 5.4%, 3.8%, 2.0%, and 1.7%, and 8.6%, 6.7%, 2.8%, and 1.9%, respectively; the difference between I2 and M for N1 and N2 was significant. Increasing N applications can also enhance root DM of maize at the maturity stage; the difference in root DM of the same cropping pattern (M, I1, or I2) between N2 and N1 and N3 and N2 was significant ($P < 0.05$), while it was not significant between N4 and N3.



Values are means ± standard deviation (n = 3). Different letters above columns indicate difference for treatments of four N levels and three cropping patterns according to LSD tests (ANOVA) at the 5% level (N fertilization $P < 0.01$; Cropping pattern $P < 0.01$; N fertilization × Cropping pattern P = 0.4488).

M: maize monoculture; I1: maize-cotton intercrop; I2: maize-soybean intercrop; N1: 100 kg ha^{-1}; N2: 200 kg ha^{-1}; N3: 300 kg ha^{-1}; N4: 400 kg ha^{-1}.

Figure 2. Effects of intercropping and N fertilization on root bleeding sap rate of maize at the filling stage.



Values are means ± standard deviation (n = 3). Different letters above columns indicate difference under treatments of four N levels and three cropping patterns according to LSD tests (ANOVA) at the 5% level (N fertilization $P < 0.01$; Cropping pattern $P < 0.05$; N fertilization × Cropping pattern P = 0.8989).

M: maize monoculture; I1: maize-cotton intercrop; I2: maize-soybean intercrop; N1: 100 kg ha^{-1}; N2: 200 kg ha^{-1}; N3: 300 kg ha^{-1}; N4: 400 kg ha^{-1}.

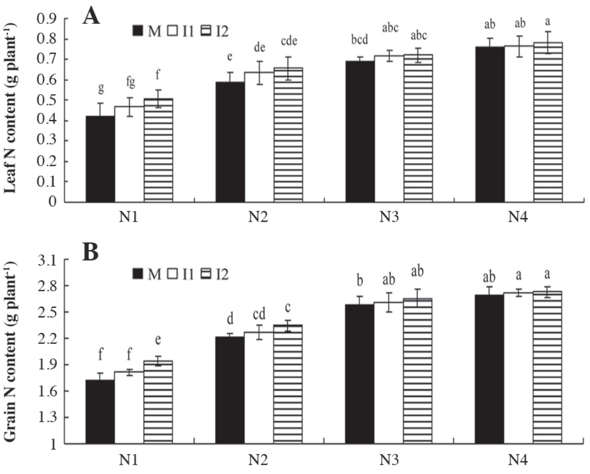
Figure 3. Effects of intercropping and N fertilization on root dry mass per plant in maize at the maturity stage.

The effect of intercropping and N fertilizer on increasing root DM of maize will gradually decrease with increasing N fertilization levels.

Leaf and grain N content and maize yield

Nitrogen fertilization and the cropping pattern had a significant effect on leaf and grain N content (Figure 4). When compared with the M treatment, I1 and I2 for N1, N2, N3, and N4 increased leaf N content by 11.9%, 6.8%, 4.3%, and 0.1%, and 21.4%, 11.9%, 4.3%, and 2.6%, and grain N content by 5.2%, 2.7%, 1.2%, and 1.1%, and 12.8%, 6.3%, 2.7%, and 1.5%, respectively. The difference in leaf and grain N content between intercropping and monoculture for N3 and N4 was not significant ($P > 0.05$). Increasing N applications can also enhance leaf and grain N content; the difference in leaf and grain N content from the same cropping pattern (M, I1, or I2) between N1 and N2 was significant ($P < 0.05$), while it was not significant between N3 and N4. The effect of intercropping and N fertilizer on increasing leaf and grain N content will gradually decrease with increasing N fertilization levels.

Within the same N level (N1, N2, N3, or N4), kernels per ear, 1000-kernel weight, economic yield per plant, biomass per plant, and yield of intercropped maize in 2012 and 2013 were higher than those for monoculture and the



Values are means ± standard deviation (n = 3). Different letters above columns indicate differences for treatments of four N levels and three cropping patterns according to LSD test (ANOVA) at the 5% level. (A): N fertilization $P < 0.01$; Cropping pattern $P < 0.05$; N fertilization × Cropping pattern $P = 0.8833$; (B): N fertilization $P < 0.01$; Cropping pattern $P < 0.01$; N fertilization × Cropping pattern $P = 0.5227$).
M: maize monoculture; I1: maize-cotton intercrop; I2: maize-soybean intercrop; N1: 100 kg ha^{-1}; N2: 200 kg ha^{-1}; N3: 300 kg ha^{-1}; N4: 400 kg ha^{-1}.

Figure 4. Effects of intercropping and N fertilization on (A) leaf and (B) grain N content at the maturity stage.

Table 3. Effects of intercropping and N fertilization on maize yield and its components.

	N fertilization	Cropping patterns	Kernels per ear	1000-kernel weight	Economic yield per plant	Biomass per plant	Yield
				g			kg ha^{-1}
2012	N1	M	510.90 ± 6.07e	265.53 ± 5.05h	118.63 ± 6.48g	287.57 ± 11.09f	8048.43 ± 115.68g
		I1	515.03 ± 8.34de	268.93 ± 3.56gh	122.53 ± 5.76fg	303.73 ± 9.18ef	8418.90 ± 159.41f
		I2	524.27 ± 4.51d	273.13 ± 6.24fg	129.27 ± 6.24ef	316.20 ± 9.73e	8511.83 ± 143.64f
	N2	M	540.83 ± 4.12c	279.90 ± 4.29ef	135.07 ± 6.56de	335.33 ± 12.44d	8923.53 ± 204.52e
		I1	544.63 ± 6.61c	283.07 ± 7.45de	138.23 ± 3.23cde	340.23 ± 8.53d	9137.60 ± 166.02de
		I2	549.23 ± 8.00c	288.43 ± 4.74d	140.10 ± 6.26cd	347.05 ± 13.07cd	9389.27 ± 109.11cd
	N3	M	566.63 ± 7.26b	297.20 ± 3.82c	144.37 ± 6.42bcd	359.07 ± 7.59bc	9588.03 ± 103.87bc
		I1	568.97 ± 8.40b	301.87 ± 6.62bc	147.23 ± 4.57abc	365.77 ± 7.74ab	9681.87 ± 183.90ab
		I2	570.73 ± 1.50b	301.60 ± 6.49bc	150.87 ± 3.99ab	371.57 ± 6.14ab	9777.37 ± 118.75ab
	N4	M	582.87 ± 7.56a	308.27 ± 5.58ab	152.03 ± 3.67ab	373.77 ± 10.78ab	9790.57 ± 219.08ab
		I1	584.33 ± 5.04a	310.07 ± 6.11a	154.93 ± 6.07a	375.40 ± 9.45a	9874.37 ± 111.70a
		I2	586.23 ± 6.26a	311.93 ± 6.23a	156.03 ± 6.92a	376.83 ± 8.95a	9924.43 ± 138.72a
Significance LSD multiple range tests (P values)							
N fertilization			0.0001	0.0001	0.0001	0.0001	0.0001
Cropping pattern			0.0426	0.0052	0.0407	0.0066	0.0003
N fertilization × Cropping pattern			0.8748	0.8657	0.9673	0.4875	0.3800
2013	N1	M	508.10 ± 13.51h	262.77 ± 5.92g	113.43 ± 5.16g	288.17 ± 17.92f	8002.47 ± 101.41i
		I1	513.53 ± 6.66gh	267.17 ± 6.86fg	118.13 ± 8.41g	303.73 ± 9.27ef	8332.93 ± 102.96h
		I2	523.93 ± 8.02fg	275.67 ± 6.95ef	127.20 ± 8.58f	322.90 ± 10.97de	8577.00 ± 96.15g
	N2	M	535.27 ± 6.66ef	280.50 ± 5.82de	132.90 ± 6.66ef	333.47 ± 13.31cd	8850.20 ± 145.69f
		I1	539.73 ± 9.06e	284.00 ± 6.35cde	138.20 ± 6.78de	342.97 ± 10.43bc	9095.43 ± 94.88e
		I2	546.97 ± 9.58de	288.13 ± 6.33cd	142.20 ± 6.45cd	344.10 ± 11.32bc	9286.13 ± 116.18d
	N3	M	560.77 ± 7.12cd	292.40 ± 6.37bc	144.53 ± 8.30bcd	356.40 ± 9.79ab	9567.97 ± 103.76c
		I1	565.57 ± 12.87bc	299.17 ± 7.89ab	147.20 ± 11.15abc	364.10 ± 9.73a	9672.03 ± 90.29bc
		I2	567.13 ± 6.67abc	301.70 ± 3.21ab	150.53 ± 9.27abc	367.17 ± 7.72a	9711.67 ± 109.77abc
	N4	M	578.70 ± 9.76ab	306.77 ± 4.08a	151.07 ± 9.25ab	368.23 ± 10.81a	9740.07 ± 120.94abc
		I1	580.67 ± 11.19ab	307.80 ± 2.65a	152.87 ± 10.96ab	369.77 ± 13.56a	9858.40 ± 112.42ab
		I2	582.03 ± 7.20a	308.20 ± 6.32a	154.77 ± 11.21a	370.37 ± 8.15a	9873.00 ± 105.11a
Significance LSD multiple range tests (P values)							
N fertilization			0.0001	0.0001	0.0001	0.0001	0.0001
Cropping pattern			0.0681	0.0147	0.0028	0.0202	0.0001
N fertilization × Cropping pattern			0.9258	0.7530	0.7224	0.3813	0.0620

Values are means ± standard deviation (n = 3). Means followed by different letters in the same column indicate differences according to LSD multiple range tests ($P < 0.05$).
M: maize monoculture; I1: maize-cotton intercrop; I2: maize-soybean intercrop; N1: 100 kg ha^{-1}; N2: 200 kg ha^{-1}; N3: 300 kg ha^{-1}; N4: 400 kg ha^{-1}.

difference between I2 and M for N1 was significant ($P < 0.05$, Table 3). When compared with the M treatment, I2 for N1, N2, N3, and N4 increased yield in 2012 by 5.8%, 5.2%, 2.0%, and 1.4%, and in 2013 by 7.2%, 4.9%, 1.5%, and 1.4%, respectively. The results of the 2 yr also showed that increasing N applications can enhance kernels per ear, 1000-kernel weight, economic yield per plant, biomass per plant, and yield of maize; the difference in yield of the same cropping patterns (S, I1 or I2) between N2 and N1 and N3 and N2 was significant, while it was not significant ($P > 0.05$) between N4 and N3. The effect of intercropping and N fertilizer on increasing maize yield will gradually decrease when the N fertilization level increases.

DISCUSSION

The advantageous effect of intercropping has been confirmed by many experiments (Zuo et al., 2000; Peng et al., 2009; Hinsinger et al., 2011). In this study, when compared with the M treatment, I1 and I2 for N1, N2, N3, and N4 increased green leaf area per plant by 3.8%, 2.5%, 1.7%, and 0.5%, and 6.1%, 5.0%, 2.7%, and 0.7%, respectively; this indicates that intercropping also has an advantageous effect on increasing green leaf area per plant in maize at the maturity stage, but its effect will gradually decrease when the N fertilization level increases. Intercropping can increase green leaf area per plant in maize mainly because one plant species enhances the survival, growth, or fitness of another in the intercropping system (Hauggaard-Nielsen and Jensen, 2005; Zhang et al., 2013). Zuo et al. (2000) indicated that rhizosphere interactions in a peanut-maize intercropping system could improve Fe nutrition of peanut and enhance its chlorophyll content. In this study, we not only found that intercropping and increasing N applications could increase chlorophyll content of maize at different growth stages, but we also found that their effects would gradually decrease with increasing N fertilization levels. Chu et al. (2004) also observed that chlorophyll content of rice leaves under intercropping conditions on 8 August and 15 September were 44.3 and 42.8 (SPAD) compared with 38.5 and 30.9 under monocropping conditions, respectively. Improving photosynthetic characteristics has a significant impact on crop yield, growth, and development (Anten, 2005). In the present study, we found that intercropping and increasing N applications could improve the photosynthetic characteristics of maize, but their effects would gradually decrease when the N fertilization level increases. In this experiment, the increase of maize leaf photosynthetic rate was mainly due to the difference in plant height between intercrops, which significantly improved the ventilation and light conditions of the maize population.

Structural and functional characteristics of roots have a significant influence on crop yield and the capacity of roots to acquire nutrients (Richardson et al., 2009; Miyazawa et al., 2010). In the present study, intercropping and increasing N applications can increase root bleeding sap rate (an index of root activity) and root DM, but their effects will gradually decrease when the N fertilization level increases. Miyazawa et al. (2010) also found that sorghum under intercropping grew deeper roots with greater biomass than under sole cropping. Intercropping can increase root bleeding sap and root DM of maize mainly because above-ground and below-ground (root) interactions in the intercropping system can improve rhizosphere soil enzyme activities and microbial environment (Sun et al., 2011; Hinsinger et al., 2011), physical and chemical properties (Wang et al., 2005; Richardson et al., 2009), and the crop's growth and development status (Hauggaard-Nielsen and Jensen, 2005; Zhang et al., 2010). Nitrogen plays an important role in crop growth and the development process; in the present study, we found that intercropping and increasing N applications could enhance leaf and grain N content of maize at the maturity stage, but their effects would decrease when the N fertilization level increased. Li et al. (2001) also indicated that N uptake by intercropping of wheat and maize was greater than the corresponding sole cropping under the same N supply level. Intercropping can increase leaf and grain N content of maize mainly because (1) interspecific interactions in the rhizosphere facilitate N uptake in intercropping systems (Li et al., 2003; Richardson et al., 2009), and (2) legumes can transfer fixed N to intercropped cereals during their joint growing period, this N is an important resource for the cereals (Li et al., 2009; Rivest et al., 2010). Li et al. (1999) found that total biomass and grain yields in a field study of intercropped maize and faba bean (*Vicia faba* L.) were significantly higher than those of maize and faba bean in the corresponding sole crops. In our 2-yr field experiment, we found that yields of intercropped maize in 2012 and 2013 were higher than for monoculture, and the difference between I2 and M for N1 and N2 was significant. We also found that the effects of intercropping and increasing N applications would decrease when the N fertilization level increased. As abovementioned, intercropping can significantly enhance crop yield in N1 mainly because intercropping can obviously help to improve green leaf area per plant, chlorophyll content, photosynthetic rate, root biomass, and yield components of a crop with a low N fertilization level. Intercropping did not significantly affect yield in N3 and N4 mainly because the increasing N applications improved crop growth and development, which aggravated interspecific competition between intercrops; the advantageous effects of intercropping to improve crop physiological traits, yield components, and soil biochemical characteristics can be partially inhibited under high N fertilization levels.

CONCLUSIONS

Given that the advantageous effect of intercropping and increasing N applications to improve crop physiological characters and increasing crop yield will gradually decrease or be partially inhibited by increasing the N fertilization level, we should pay attention to adopting reasonable cropping patterns in agricultural production practices, increase crop yield by enhancing crop fertilizer use efficiency, and making better use of niche complementarity and interspecific facilitation of intercrops rather than by a high fertilizer input rate.

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