

Effect of *Orychophragmus violaceus* incorporation on nitrogen uptake in succeeding maize

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ABSTRACT

Winter *Orychophragmus violaceus* (OV)/spring maize is a novel eco-agricultural system in North China Plain, but little is known about OV's nitrogen (N) effects on succeeding maize growth and its contribution to maize N uptake and utilization during the growth. A pot experiment using ¹⁵N was conducted, including five treatments: (1) Control – without OV incorporation and N fertilizer; (2) G – high ¹⁵N labelled-OV incorporation; (3) 50%G – lower ¹⁵N labelled-OV incorporation; (4) F – ¹⁵N labelled urea fertilizer merely; (5) G + F – high OV incorporation combined with urea fertilizer. Increasing OV incorporation rate increased maize dry matter, yield, and total N uptake. Approximately 84–97% of the released OV's nitrogen was absorbed before the V8 (the 8th leaf fully expanded) stage in G and 50%G treatments. However, only 19% of the released OV's nitrogen was taken up at this period in G + F, and the rest was absorbed from V8 to maturity. G treated maize doubled the OV's nitrogen uptake than the 50%G at maturity, and also had higher percentage contribution of OV's N to total N accumulation. The G and 50%G maize obtained similar OV's nitrogen use efficiency of 30%; however, this efficiency was further improved to 43% in the G + F. And more, G + F treatment further improved the maize yield by 9% compared to F treatments.

Keywords: green manure; nitrogen sources; *Zea mays* L.; cover crops

Orychophragmus violaceus L. (OV)/spring maize (*Zea mays* L.) production system is an innovative eco-agricultural practice, developed to secure crop cover of the increasing winter fallow area in North China Plain, through growing OV winter cover crops (Liu et al. 2010, 2012). In the temperate zone, winter cover crops are usually grown to catch available nitrogen (N) in the soil and thereby prevent N leaching losses, or to improve the nutrition of the subsequent main crops (Wyland et al. 1996, Thorup-Kristensen et al. 2003, Hooker et al. 2008). After the winter period, the nitrate content in OV covered soil was lower than the fallow soil (Xiong et al. 2014). OV, belonging to the Brassicaceae plant family, could partially function as catch crop which reduces N leaching by taking up residual N

from the soil, as other crucifers have been found to have extensive and deep root growth (Thorup-Kristensen 2001, Thorup-Kristensen et al. 2003). Soil N depletion was highly correlated to crucifer crops rooting depth (Thorup-Kristensen 2001). Meanwhile after incorporation, OV decomposition produced more nitrate than control in 0–100 cm soil layers, especially in 0–20 cm which increased the soil mineral nitrogen content (Xiong et al. 2014). Due to the changes of soil temperature, water moisture, and porosity etc., after incorporation, OV plus N fertilizer led to similar or slightly higher NH₃ volatilization (averagely 1.04 kg/ha) than the sole fertilizer usage during maize growth (Xiong et al. 2013). These results indicated that, after OV incorporation, there is a risk of N loss in the OV-

spring maize production system. It quite needs to investigate the OV's N effects and contribution to the succeeding maize N uptake at different stages.

It is often expected that cover crops supply N for the succeeding plants. Different kinds of cover crops and their N effects on succeeding maize production were examined (Vyn et al. 1999, Astier et al. 2006, Tosti et al. 2012). For the complex interaction between the characteristics of cover crop, the soil, and the climate etc., cover crop could increase or decrease the N supply for a succeeding crop (Thorup-Kristensen et al. 2003, and references therein), thus playing an important role in subsequent crop nitrogen uptake, yield increase and stability. OV incorporation increased the soil N availability, as well as N uptake and grain yield of succeeding maize (Yang et al. 2013). To this extent, OV acts as green manure to improve soil fertility and supply nutrients for following crop growth. It is also regarded as a biological approach to sustain the ecosystem function and production (Thorup-Kristensen et al. 2003).

The growth and nutritional characteristics of OV, and its effects on N losses have been well studied in our previous work (Liu et al. 2010, 2012, Xiong et al. 2013, 2014), but there is still a lack of information on the OV's N effects on succeeding maize growth and yield, its contribution to N uptake of maize and utilization efficiency of different N sources after OV incorporation.

MATERIAL AND METHODS

The pot experiment was conducted in 2012 at the Wanzhuang Experimental Station, Chinese Academy of Agricultural Sciences, China. The soil type was calcareous sandy soil and its chemical properties were as follows: N_{\min} (NO_3^- -N + NH_4^+ -N) 7.7 mg/kg, P_{Olsen} 6.9 mg/kg, K_{NH_4OAc} 65.2 mg/kg, organic matter 5.31 g/kg, and pH (H_2O) 8.87. A current maize cv. ZD958 was employed in this study. The incorporated green manure was *Orychophragmus violaceus* L. To get the ^{15}N enriched OV plants, 0.79 g ^{15}N was applied at OV sowing and fast growing stage, respectively, in a 2.6 m² microplot. Simultaneously, the unlabeled N was applied at the same rate and time in a distant microplot to get non-labeled OV.

To quantify the N uptake in maize derived from OV, urea fertilizer, and soil, five treatments with four rep-

licates were set in a completely randomized design: (1) Control – no urea and OV incorporation; (2) G – 200 g fresh weight (FW) ^{15}N labelled OV incorporation (^{15}N atm% = 2.02%); (3) 50%G; (4) F – ^{15}N labelled urea (^{15}N atm% = 10.30%, the Shanghai Research Institute of Chemical Industry, Shanghai, China); (5) G + F – combination of OV and fertilizer N, consisted of two sub-treatments, i.e. labeled OV plus unlabeled urea (^{15}G + F), and unlabeled OV plus labeled urea (G + ^{15}F). The collected OV plants were cut into about 1 cm pieces and mixed with the whole volume of pot soil at 10 days before maize sowing, and fertilizer was applied as urea, super phosphate and potassium sulphate. The N supply of the five treatments was 0, 0.47, 0.94, 2.00, and 2.94 g per pot containing 10 kg air dried soil, respectively. All treatments were supplied with 0.44 g P per pot, and 0.83 g K per pot. Besides that, 50%G was additionally introduced with 0.043 g P and 0.46 g K per pot, as well as 0.096 g P and 0.92 g K per pot in G and G + F treatments. The soil moisture was controlled within a range from 9.6–11.2%, at corresponding to 60–70% of water holding capacity during the maize growing.

Two maize seeds were sown in each pot, and thinned to one plant around the second fully expanded leaf. Maize roots and shoots were sampled at V3 (the 3rd leaf fully expanded), V8 (the 8th leaf fully expanded), silking, and physiological maturity, and the shoot was further divided into leaves, stem, cob plus husks, and kernels (when applicable). Samples were dried at 70°C and weighed. The plant tissue $^{14}N/^{15}N$ assay, as well as soil samples at maturity, was conducted using an isotope ratio mass spectrometer (Finnigan Delta Plus XP, Thermo Electron Corporation, Bremen, Germany).

The calculation in the present study was based on the assumption that N uptake in maize is only derived from soil, OV incorporation, and urea application. For each plant organ, termed x :

$$GN = TN \times (Ax\% - A_0x\%) / (A_G\% - 0.3668) \times 100$$

$$FN = TN \times (Ax\% - A_0x\%) / (A_F\% - 0.3668) \times 100$$

$$SN = TN - GN - FN$$

Where: GN, FN, and SN – N uptake in organ x derived from green manure OV, urea fertilizer, and soil; TN – total N uptake in organ x ; $A_G\%$, $A_F\%$ – ^{15}N abundance of labeled OV and labeled urea, i.e. 2.02% and 10.30%; $Ax\%$, $A_0x\%$ – ^{15}N abundance in the organ x from labeled and unlabeled maize.

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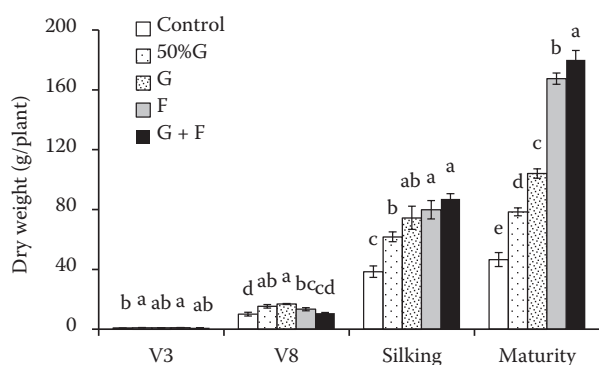


Figure 1. The whole plant dry weight of maize at different stages ($n = 4$). Different letters above the column represent significant differences among treatments ($P < 0.05$). Control – without *Orychophragmus violaceus* (OV) incorporation and nitrogen (N) fertilizer; G – high ^{15}N labelled-OV incorporation; 50%G – lower ^{15}N labelled-OV incorporation; F – ^{15}N labelled urea fertilizer merely; G + F – high OV incorporation combined with urea fertilizer; V3 – the 3rd leaf fully expanded; V8 – the 8th leaf fully expanded

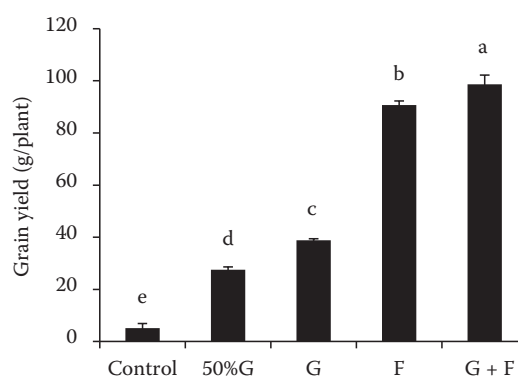


Figure 2. The maize grain yield of different treatments at maturity ($n = 4$). Different letters above the column represent significant difference among treatments ($P < 0.05$). Control – without *Orychophragmus violaceus* (OV) incorporation and nitrogen (N) fertilizer; G – high ^{15}N labelled-OV incorporation; 50%G – lower ^{15}N labelled-OV incorporation; F – ^{15}N labelled urea fertilizer merely; G + F – high OV incorporation combined with urea fertilizer

NUE – the ratio of the amount of ^{15}N recovered in plant to the amount of ^{15}N applied.

The statistical significance of differences among treatments were tested with ANOVA (SAS software package 8.02, SAS Institute, Cary, USA); and the least significant difference (LSD) was used to compare the means.

RESULTS AND DISCUSSION

OV incorporation effects on maize biomass and grain yield. Both at maize V3 and V8 stages, the 50%G

and G had similar or even higher biomass than F and G + F maize (Figure 1), indicating that two pure OV treatments could sustain the early vegetative growth of maize. At maturity, increasing OV incorporation rate increased maize dry weight (DW) by 33% (50%G vs. G, Figure 1) and grain yield by 41% (Figure 2). OV plus urea further boosted the whole plant DW by 7.5% (Figure 1) and grain yield by 9% (Figure 2) compared to F. These results showed that green manure had a significant effect on maize growth and grain yield, mainly by increasing the soil N availability for succeeding crops (Astier et al. 2006, Tosti et al. 2012, Yang et al. 2013).

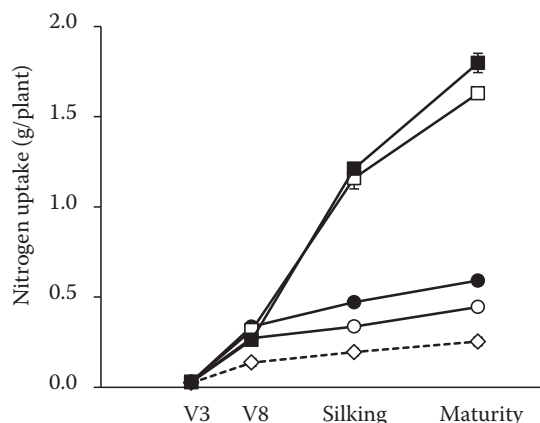
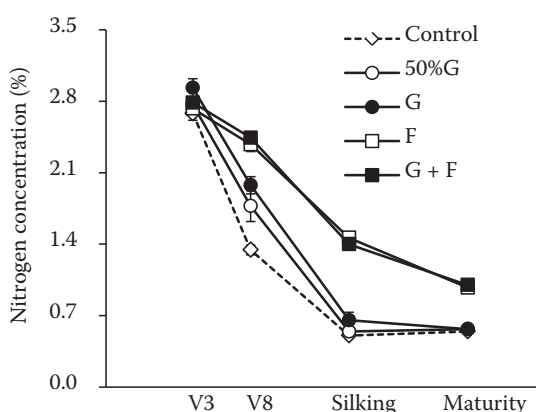


Figure 3. The whole plant nitrogen (N) concentration and uptake in maize at different stages ($n = 4$). Control – without *Orychophragmus violaceus* (OV) incorporation and N fertilizer; G – high ^{15}N labelled-OV incorporation; 50%G – lower ^{15}N labelled-OV incorporation; F – ^{15}N labelled urea fertilizer merely; G + F – high OV incorporation combined with urea fertilizer; V3 – the 3rd leaf fully expanded; V8 – the 8th leaf fully expanded

Table 1. The nitrogen (N) uptake (mg/plant) from different sources, and its percentage to total N uptake at each growth interval during maize growth with different treatments

N source	Treatment	Sowing–V3		V3–V8		V8–Silking		Silking–maturity		Sowing–maturity	
		(mg/plant)	(%)	(mg/plant)	(%)	(mg/plant)	(%)	(mg/plant)	(%)	(mg/plant)	(%)
G-N	50%G	17 ^b	55	120 ^b	50	1 ^c	1	4 ^b	4	142 ^c	32
	G	22 ^a	74	210 ^a	69	40 ^b	30	4 ^b	3	276 ^b	47
	G + F	7 ^c	25	69 ^c	29	235 ^a	25	97 ^a	17	408 ^a	23
F-N	F	22 ^a	73	205 ^a	71	636 ^a	76	310 ^a	66	1173 ^a	72
	G + F	15 ^b	55	131 ^b	56	597 ^a	63	349 ^a	59	1092 ^a	61
S-N	control	24 ^a	100	113 ^{ab}	100	58 ^c	100	59 ^c	100	254 ^c	100
	50%G	13 ^b	45	121 ^a	50	65 ^c	99	104 ^b	96	304 ^b	68
	G	8 ^c	26	96 ^{ab}	31	95 ^{bc}	70	116 ^b	97	315 ^b	53
	F	8 ^c	27	82 ^b	29	207 ^a	25	160 ^a	34	457 ^a	28
	G + F	5 ^d	20	35 ^c	15	118 ^b	12	142 ^{ab}	24	300 ^b	17

G-N, F-N, S-N – nitrogen derived from *Orychophragmus violaceus* (OV), urea, and soil. Values followed by different letters in the same column within the same N sources represent significant differences among treatments ($P < 0.05$). Control – without OV incorporation and N fertilizer; G – high ¹⁵N labelled-OV incorporation; 50%G – lower ¹⁵N labelled-OV incorporation; F – ¹⁵N labelled urea fertilizer merely; G + F – high OV incorporation combined with urea fertilizer; V3 – the 3rd leaf fully expanded; V8 – the 8th leaf fully expanded

Nitrogen uptake, sources, and utilization in maize plants. 50%G and G treated maize took up as much as or more N than the F and G + F treatments at V3 and V8, indicating that the differences in growth and in N uptake among these treatments were not solely related to the N supply. But after V8, the 50%G and G plants suffered a larger reduction of N concentration than F and G + F maize, and the N uptake was also left behind after silking (Figure 3) for lesser N availability in the soil. At maturity, G treated maize increased total N

uptake by 32% than 50%G, but both just accounted for 25–36% of N uptake in F and G + F maize. Again, the G + F further enhanced N uptake by 10% compared to F (Figure 3), which highlighted the complementary function of the organic and inorganic N supplies (Cherr et al. 2006).

Table 1 showed the N sources and their percentage contribution to the total N accumulation in maize plants at different growth periods. As to N derived from OV, G treated maize took up more OV's N than 50%G before silking, and 84% and

Table 2. Green manure nitrogen (G-N) and fertilizer N (F-N) accumulation in maize organs and their distribution at maturity

Organ	G-N						F-N			
	accumulation (mg/plant)			distribution (%)			accumulation (mg/plant)		distribution (%)	
	50%G	G	G + F	50%G	G	G + F	F	G + F	F	G + F
Root	13 ^b	22 ^a	23 ^a	9	8	6	44 ^a	51 ^a	4	5
Stem	13 ^b	27 ^a	30 ^a	9	10	8	80 ^a	78 ^a	7	7
Leaves	26 ^b	45 ^a	42 ^a	18	16	10	116 ^a	103 ^a	10	9
Cob + Husks	12 ^c	23 ^b	34 ^a	8	8	8	92 ^a	87 ^a	8	8
Grain	78 ^c	159 ^b	278 ^a	55	58	68	841 ^a	772 ^b	72	71

Values followed by the different letters in the same row within the same N sources represent significant differences among treatments ($P < 0.05$). G – high ¹⁵N labelled-OV incorporation; 50%G – lower ¹⁵N labelled-OV incorporation; F – ¹⁵N labelled urea fertilizer merely; G + F – high OV incorporation combined with urea fertilizer

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Table 3. ^{15}N , derived from green manure (G-N) and/or urea fertilizer (F-N), use efficiency of maize with different treatments

Treatment	Nitrogen sources	N input (g/pot)	N uptake (mg/plant)	N use efficiency (%)	Residue (mg/pot)	Residue rate (%)	Losses (mg/pot)	Loss rate (%)
50%G	G-N	0.47	142 ^f	30 ^d	192 ^d	41 ^a	136 ^e	29 ^b
G	G-N	0.94	276 ^e	29 ^d	317 ^b	34 ^b	347 ^c	37 ^a
F	F-N	2.00	1173 ^b	59 ^a	253 ^c	12 ^f	575 ^b	29 ^b
G + F	G-N	0.94	408 ^d	43 ^c	271 ^c	29 ^c	261 ^d	28 ^b
	F-N	2.00	1090 ^c	55 ^{ab}	332 ^b	17 ^e	575 ^b	29 ^b
	G-N plus F-N	2.94	1499 ^a	51 ^b	603 ^a	21 ^d	838 ^a	29 ^b

Values followed by the different letters in the same column represent significant differences among treatments ($P < 0.05$). Control – without *Orychophragmus violaceus* (OV) incorporation and N fertilizer; G – high ^{15}N labelled-OV incorporation; 50%G – lower ^{15}N labelled-OV incorporation; F – ^{15}N labelled urea fertilizer merely; G + F – high OV incorporation combined with urea fertilizer

97% of the released OV's N uptake occurred by V8 in the two pure OV treatments respectively, implying the quick decomposition of incorporated cover crops (Breland 1994, Dou et al. 1994, Thorup-Kristensen et al. 2003). However, only 19% of the released OV's N was taken up by V8 in G + F treatment, and the rest was absorbed from V8 to maturity, when plants grow fast and large amounts of water and nutrients are needed, indicating that green manure N uptake could potentially last longer for uptake at later stages when combined with fertilizer N application. At maturity, OV's N uptake was doubled with the incorporation rate increasing from 50%G to G, and was further enhanced by G + F treatment (Table 1). The superiority of the combination of OV and fertilizer might be related to the following aspects: (1) pool substitution, where fertilizer N is used by microorganisms during decomposition, meaning that they use less green manure N and more is therefore available for the maize plants. (2) More N in the soil promotes the decomposition of the green manure, and therefore more of the green manure N is released (Thorup-Kristensen 1994), for the OV material holding lower C/N ratio around 13. As to N derived from urea, G + F treated maize accumulated less fertilizer N than F from sowing to V8, and the difference was offset by the OV's N uptake (Table 1), implying the potential of fertilizer replacement by green manure.

The partitioning of N from different sources was further observed to investigate their utilization within the maize plants (Table 2). G treatment increased the OV's N uptake in each plant organ

over 50%G, but did not affect the partitioning percentages (Table 2). While compared to G, G + F maize had similar OV's N uptake in vegetative organs, but more in the ears especially in grain (278 vs. 159 mg/plant), consequently the higher partitioning percentage to grains (68% vs. 58%). Subedi and Ma (2005) reported that when N was supplied later during the development of maize, a higher proportion of uptake into grains, which was confirmed by Seebaur et al. (2010), implying longer duration of OV's N for later uptake in G + F in present study.

Nitrogen use efficiency. Increasing OV incorporation rate from 50%G to G led to a two-fold increase of the OV's N uptake (Tables 1 and 3), resulting in a similar N recovery of 30%. While in the G + F treatment, OV's N use efficiency was significantly improved to 43%, which might be partially associated with more OV's N uptake in G + F where the decomposition and mineralization of OV materials was promoted by the additional fertilizer N supply. Alternatively it might be also related to significantly lower loss rate (28.8%) than that in G treatment (36.9%, Table 3). The recovery of total applied N, derived from both OV and urea, was up to 51% in G + F treatments, which however, was still significantly lower than the total NUE in F treatment (Table 3). This decrease was partly associated with the decreased urea N uptake due to OV incorporation and replacement, which indicated that for optimal nitrogen use efficiency, the N fertilizer application rate should be somewhat reduced in the OV systems, to compensate for the N supplied by mineralization of the OV material.

In conclusion, OV and fertilizer N combination improved the green manure N uptake in maize, leading to an increased green manure N use efficiency, compared to sole OV incorporation. What's more, it significantly increased the succeeding maize yield and whole plant N uptake compared to sole urea application. We suggest that OV/spring maize production system in NCP could hold great promise to deal with reducing fertilizer usage by replacement and sustaining crop yield.

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