Effects of long-term fertilization on leaf photosynthetic characteristics and grain yield in winter wheat

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Abstract

Based on a 20-year fertilization experiment with wheat-maize double cropping system, the effects of different long-term fertilization treatments on leaf photosynthetic characteristics and grain yield in different winter wheat (*Triticum aestivum* L.) cultivars were studied in the growing seasons of 2000–2001 and 2001–2002. A total of nine fertilization treatments were implemented, *i.e.* no fertilizer (CK), N fertilizer (N), N and P fertilizers (NP), N and K fertilizers (NK), N, P, and K fertilizers (NPK), only organic manure (M), organic manure and N fertilizer (MN), organic manure and N and P fertilizers (MNP), and organic manure and N, P, and K fertilizers (MNPK). With the treatments of combined organic manure and inorganic fertilizers (TMI), net photosynthetic rate (P_N), maximal activity of photosystem 2, PS2 (F_v/F_m), and chlorophyll content (SPAD value) of flag leaves and leaf area index (LAI) were much higher at the mid grain filling stage (20 or 23 d post anthesis, DPA), and exhibited slower declines at the late grain filling stage (30 DPA), compared with the treatments of only inorganic fertilizers (TI). The maximal canopy photosynthetic traits expressed as P_N×LAI and SPAD×LAI at the mid grain filling stage were also higher in TMI than those in TI, which resulted in different grain yields in TMI and TI. Among the treatments of TMI or among the treatments of TI, both flag leaf and canopy photosynthetic abilities and yield levels increased with the supplement of inorganic nutrients (N, P, and K fertilizers), except for the treatment of NK. Under NK, soil contents of N and K increased while that of P decreased. Hence the unbalanced nutrients in soil from the improper input of nutrients in NK treatment were probably responsible for the reduced flag leaf and canopy photosynthetic characteristics and LAI, and for the fast declining of flag leaf photosynthetic traits during grain filling, resulting in the reduced yield of NK similar to the level of CK.

Additional key words: chlorophyll; chlorophyll *a* fluorescence; N, P, and K fertilizers; leaf area index; net photosynthetic rate; organic manure; photosystem 2; *Triticum aestivum* L.

Introduction

Since the first long-term fertilizer experiment initiated by Lawes and Gilbert at the Rothamsted Experiment Station, UK in 1843, a series of long-term fertilization experiments were set up to study the effects of long-term fertilization on the soil properties, fertility, nutrient balance and productivity, nutrient use efficiency, and grain yield and quality of crops (Wadman 1987, Fraser *et al*. 1988, Reganold *et al.* 1993, Liebig and Doran 1999). Valuable information and evidence obtained from these studies contributed greatly to the modern theory and practice for crop fertilization (Edmeades 2003). Since 90s of last century, many of these researches turned to the evaluation on runoff and leaching of nutrients under long-term fertilization (Sharpley *et al.* 1994, Jarvis *et al.* 1995, Gillingham and Thorrold 2000, Ledgard *et al.* 2000), and to the soil quality (Liebig and Doran 1999). Many reviews detailing these studies have been published (Jenkinson 1991, Edmeades 2003).

In China, the first long-term fertilization experiment was started in 1950 (Tian 2002), but countrywide large-scale experiments were established in the late 1970s to early 1980s to study the effects of long-term fertilization on physical and chemical properties of soil, grain yield, and quality of crops (Wang *et al.* 1995, Zhang *et al.* 1997, Tian 2002). As influenced by the conventional crop management practices, the long-term fertilization experiments in China exhibited some special features, especially in the aspects of typical double or triple cropping

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systems and combined application of organic and inorganic fertilizers. Thus, in most cases outside of China, the effect of single manure or inorganic nutrient was compared while in China the benefit of combined organic and inorganic fertilizers was intensively illustrated (Zeng *et al.* 1992, Zhou *et al.* 2002).

Most previous studies with long term fertilization have focused on the physical and chemical properties and quality of soil, and on yield and quality of crops. Reports on the effects of long-term fertilization on physiological and biochemical characteristics of crop plants are very limited. Therefore, we tried to elucidate the effects of long-term fertilization on leaf photosynthetic characteristics and grain yield in winter wheat, based on a 20 years' fertilization experiment with wheat-maize double cropping system in China. The results would help characterize the photosynthetic mechanism for changed grain yield under long-term fertilization and improve fertilization strategy in modern wheat production systems in China.

Materials and methods

The experiment was conducted in the Xuzhou Institute of Agricultural Sciences, Xuzhou (34ºN, 117ºE), Jiangsu Province, China. The original experiment was initiated from autumn in 1980, with the sowing of wheat. The soil was a yellow fluvo-aquic soil with sandy texture, contained organic matter of 10.8 g kg^{-1}, total nitrogen of 0.66 g kg^{-1}, available P of 12 mg kg^{-1}, and available K of 63 mg kg^{-1}. In each year, wheat-maize double cropping system was maintained, and at each cropping season, the fixed fertilization treatments were implemented in the same plots. The experiment was a two-factorial split plot design with four replications and each plot size of 33.3 m^2. The main-factor was organic manure application with two levels: applying and not applying organic manure. The sub-factor was inorganic fertilizer with four levels: no fertilizer (CK), N fertilizer (N), N and P fertilizers (NP), N, P, and K fertilizers (NPK). After 15 years, *i.e.* in 1995, two replications of the N treatment were re-designed as application of N and K fertilizers (NK). For organic manure, 3.75 kg m^{-2} horse residue that commonly contained 7.21 g(N) kg^{-1}, 4.78 g(P_2O_5) kg^{-1}, and 9.02 g(K_2O) kg^{-1} was used from 1981 to 1984, while 1.88 kg m^{-2} cow residue that commonly contained 5.41 g(N) kg^{-1}, 5.50 g(P_2O_5) kg^{-1}, and 6.82 g(K_2O) kg^{-1} was used from 1985 till now. For inorganic fertilizers, the application amounts were 15.00 g(N) m^{-2}, 7.50 g(P_2O_5) m^{-2}, and 11.25 g(K_2O) m^{-2}.

In the growing season of 2000–2001, winter wheat cultivars Wanmai 38 and Yan 2801 were used, while in 2001–2002 Xuzhou 25, Xuzhou 26, and Yan 2801 were used. All the cultivars were sown in each of the plots, and the factor of cultivar was arranged as sub-sub factor. The nutrient contents in the soil of each treatment before sowing of 2000 and 2001 seasons are shown in Table 1. Except for the fertilization treatments, the management of wheat followed the local standard for high-yield wheat (Zhang and Zhang 2001).

In 2000–2001, net photosynthetic rate (P_N), the maximal photosystem 2 (PS2) capacity (F_v/F_m), and greenness expressed in SPAD value, which well reflects leaf chlorophyll (Chl) content of flag leaves were measured at 20 and 30 d after anthesis (DPA), while leaf area index (LAI) was measured only at 20 DPA. In 2001–2002, all these parameters were measured only at 23 DPA. At maturity, 4 m^2 of wheat was harvested and threshed to get yield of each plot. Means and standard error (S.E.) of two plots in both the N and NK treatments and of four plots in the other treatments were calculated.

P_N of flag leaf was measured with a *CI-310PS* portable photosynthesis system (*CID*, Camas, WA, USA). The photon flux (PAR) was controlled at about 1 000 μmol m^{-2} s^{-1}. After dark adaptation for 15 min, F_v/F_m of flag leaf was logged with a *CI-510CF* module of the *CI-310* photosynthesis system. SPAD of flag leaf was measured with a *Minolta SPAD-502* Chl meter (*Minolta*, Japan). The SPAD (Soil Plant Analysis Development) Chl meter measures transmission of red radiation at 650 nm and of infrared radiation at 940 nm and calculates

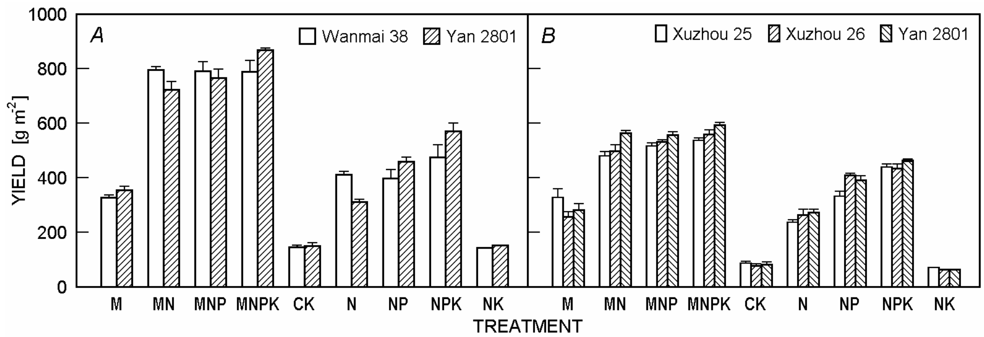

Fig. 1. Effects of long term fertilization treatments on yield of different wheat cultivars in the 2000–2001 (*A*) and 2001–2002 (*B*) seasons. Each column with a bar indicates mean + S.E. of two replications for N and NK, and of four replications for other treatments.

Table 1. Soil fertility status before sowing for different fertilization treatments in 2000 and 2001. M = manure, MN = manure + N fertilizer, MNP = manure + N and P fertilizers, NPK = manure + N, P, and K fertilizers, CK = no manure and fertilizer, N = N fertilizer, NP = N and P fertilizers, NK = N and K fertilizers, MNPK = N, P, and K fertilizers.

	2000				2001			
	Org. manure [g kg^{-1}]	N [g kg^{-1}]	Available P [mg kg^{-1}]	Available K [mg kg^{-1}]	Org. manure [g kg^{-1}]	Total N [g kg^{-1}]	Available P [mg kg^{-1}]	Available K [mg kg^{-1}]
M	19.5	1.33	71.8	75.0	20.0	1.64	69.4	73.4
MN	20.2	1.43	64.7	67.9	20.5	1.66	71.2	72.5
MNP	19.9	1.49	98.6	79.4	21.0	1.67	97.8	80.1
MNPK	20.9	1.50	98.4	95.2	21.5	1.64	101.1	101.0
CK	9.1	0.82	4.9	54.7	9.3	0.98	4.5	55.2
N	12.2	0.96	4.7	55.0	11.1	0.85	3.6	49.4
NP	13.3	0.84	12.5	52.5	11.5	1.06	11.8	48.9
NPK	12.5	0.91	13.1	65.1	12.1	1.10	13.5	66.4
NK	12.1	0.79	4.0	60.2	11.0	0.84	3.5	65.3

a value which is well correlated with Chl content (Markwell *et al.* 1995, Hoel and Solhaug 1998). The SPAD value was measured at the 1/3 to 1/2 from top of leaf. The measurement was done for five times for each leaf, and the mean was calculated as the SPAD value of the given leaf. Five flag leaves of each plot were measured for the above parameters. LAI was measured with an *AccuPAR 80* ceptometer (*Decagon Devices*, Pullman, WA, USA). The ceptometer contains 80 individual quantum sensors on the probe, which strictly measure the PAR-radiation. With readings of PAR under and above the canopy, LAI is automatically calculated according to the Beer-Lambert law by the ceptometer. For each measurement of LAI, PAR values above and under a canopy were read. Three in-row and three inter-row measurements were done for each plot, and the mean was calculated as the LAI of the given plot.

Results

Effect on grain yield: Grain yields with the treatments of combined organic manure and inorganic fertilizers (TMI) were obviously higher than those of corresponding treatments of only inorganic fertilizers applied (Fig. 1). In 2000–2001, yields with the treatments of M, MN, MNP, and MNPK were 133, 109, 81, and 59 % higher, respectively, while in 2001–2002 were 272, 101, 43, and 27 % higher, respectively, than those with CK. Yields of NK were very low and similar to those of CK in all cultivars and in both seasons. This could be related to the unbalanced nutrients in the soil from the disproportional input of nutrients with NK treatment for soil K content under NK was higher, while contents of N and P were similar, as compared with the treatment of N (Table 1). The changing patterns of yields among different treatments were similar in the two growing seasons, although the yields in the 2001–2002 season were higher than those in the 2000–2001 season, which could be caused by the arid condition in the 2000–2001 season, 30 % lower precipitation than in the 2001–2002 season.

P_N: Around mid grain filling stage, *i.e.* 20 DPA in 2000–2001 (Fig. 2*A,B*), or 23 DPA in 2001–2002 (Fig. 2*C*), P_N of flag leaf were higher in all treatments than those at 30 DPA. The differences in P_N among treatments of TMI and among treatments of TI were small, although P_N tended to be higher in treatments of TMI than in TI, and increased with supplement of N, P, and K nutrients (except for NK). Between 20 and 30 DPA in 2000–2001 (Fig. 2*A,B*), however, P_N decreased sharply, and the differences among different treatments enlarged obviously. The flag leaf photosynthetic function was finally lost with the treatments of M and CK in Wanmai 38, and then with treatment of M in Yan 2801 at 30 DPA in the 2000–2001 season. This indicated that the application of only organic manure did not essentially improve the photosynthetic capacity, compared with inorganic fertilizers. The treatments of fertilization, especially of TMI, obviously increased P_N at the late grain filling stage. The improper combination of nutrients as NK, however, did not favour the maintenance of P_N at the late grain filling stage, and thus resulted in the disability of photosynthetic function at 30 DPA.

F_v/F_m reflects the maximal PS2 capacity of leaf. The changing pattern of F_v/F_m was similar to that of P_N under different long-term fertilization treatments (Fig. 3). F_v/F_m of flag leaves under TMI was obviously higher than those under TI. At 20 or 23 DPA, the mid grain filling stage, the differences among treatments of TMI and among TI were very small, while at 30 DPA, the late grain filling stage, those differences increased greatly. F_v/F_m with the treatment of NK was similar to that of CK, especially at 30 DPA, F_v/F_m was only about 0.21 to 0.29, indicating that the photosynthetic function of flag leaf was completely disabled under NK.

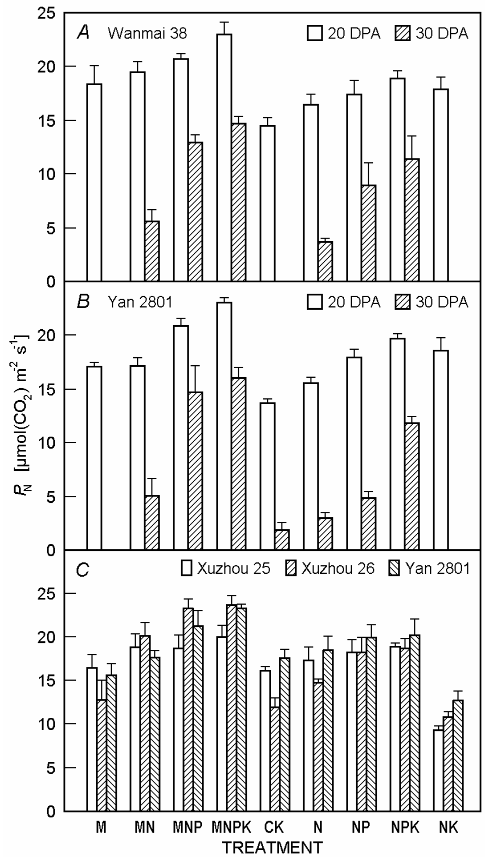

Fig. 2. Effects of long term fertilization treatments on net photosynthetic rate (P_N) of flag leaf in different wheat cultivars at 20 (*A*) and 30 (*B*) d after anthesis (DPA) in the 2000–2001 season and at 23 DPA in the 2001–2002 season (*C*). Each column with a bar indicates mean ± S.E. of two replications for N and NK, and of four replications for other treatments.

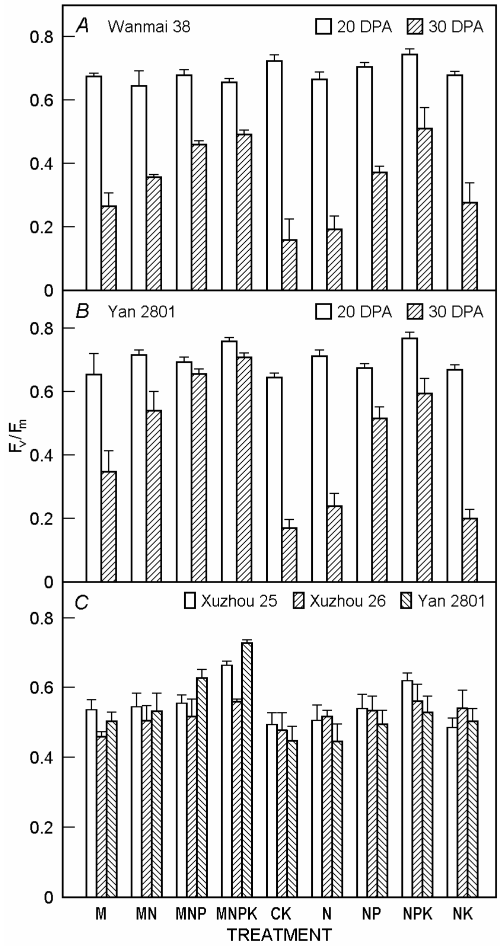

Fig. 3. Effects of long term fertilization treatments on the maximum photochemical quantum yield (F_v/F_m) of flag leaf in different wheat cultivars at 20 (*A*) and 30 (*B*) d after anthesis (DPA) in the 2000–2001 season and at 23 DPA in the 2001–2002 season (*C*). Each column with a bar indicates mean ± S.E. of two replications for N and NK, and of four replications for other treatments.

SPAD value reflecting Chl content in leaf consequently showed a similarly changing pattern as P_N (Fig. 4). In general, SPAD values of flag leaf were higher in TMI, compared with TI. Among TMI and TI, SPAD increased with the enhancement of inorganic fertilizer (except for the treatment of NK). At 20 DPA of the season 2000–2001 the differences in SPAD among treatments of TMI and among treatments of TI were small, while they enlarged greatly at 30 DPA. At 23 DPA in 2001, the changing pattern of SPAD among different fertilization treatments was similar to that at 20 DPA in 2000–2001. SPAD values of flag leaf in NK were very low and similar to those of CK with all cultivars and in both growing seasons, indicating the abnormal growth status of wheat leaves in NK, especially at the late grain filling stage.

LAI: The *AccuPAR 80* ceptometer is suitable for LAI measurement only when the green leaves account for a high proportion of the total leaf area of the plant. Thus, in the present study LAI was measured only at the mid grain filling stage (about 20 DPA). At 20 DPA in 2000–2001 or 23 DPA in 2001–2002, LAI values with treatments of TMI were obviously higher than those with TI (Fig. 5). LAI increased with the enhancement of inorganic nutrients (except for NK) in both TMI and TI. In NK, LAI was very low and similar to that of CK in all cultivars and both seasons.

Effect on canopy photosynthetic characteristics: In general, from mid to late grain filling, P_N and SPAD of flag leaf are the highest among all leaves on the same stem in wheat. Thus, the products of P_N×LAI and SPAD×LAI represent the maximum canopy photosynthetic capacity and maximum canopy leaf Chl density, respectively, thus reflecting the maximum canopy photosynthetic function in wheat. The P_N×LAI and SPAD×LAI were much higher in the treatments of TMI than those in the corresponding

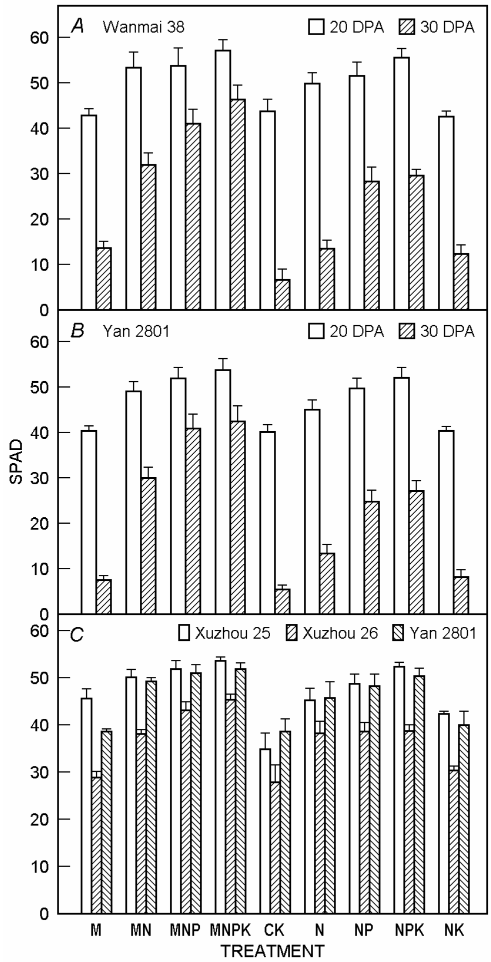

Fig. 4. Effects of long term fertilization treatments on SPAD values of flag leaf in different wheat cultivars at 20 (*A*) and 30 (*B*) d after anthesis (DPA) in the 2000–2001 season and at 23 DPA in the 2001–2002 season (*C*). Each column with a bar indicates mean + S.E. of two replications for N and NK, and of four replications for other treatments.

treatments of TI (Fig. 6), indicating that the combined organic and inorganic fertilization improved the canopy photosynthetic function at mid grain filling stage, and thus provided more substrates for grain filling in wheat. In NK, the P_N×LAI and SPAD×LAI were very low and similar to those in CK.

Correlation between grain yield and photosynthetic characteristics: Under different fertilization treatments, P_N, F_v/F_m, and SPAD of flag leaf as well as LAI were positively related to grain yields in wheat (Fig. 7). The canopy photosynthetic function parameters, P_N×LAI, and SPAD×LAI, were significantly related to grain yield, with higher correlation coefficients than those of single leaf photosynthetic parameters as P_N, F_v/F_m, and SPAD. Therefore, the much higher LAI, P_N×LAI, and SPAD×LAI could at least partly explain the higher yield in TMI than in TI. The decreased photosynthetic capacities of both single leaf (P_N, F_v/F_m, and SPAD) and canopy (LAI, P_N×LAI, and SPAD×LAI) could be important factors for causing the very low yield with high input of only N and K fertilizers in the NK treatment.

Discussion

In previous long-term fertilization experiments, fertilization of either organic manure or inorganic fertilizers improved grain yield to a great extent (Edmeades 2003), which was supported by the results in the present study. However, we found that after continuously applied N fertilizer for fifteen years and then continuously applied N and K fertilizers (NK), grain yield was reduced to the level similar to that of CK. The K content in soil was higher in treatment of NK than that in the treatment of N, while contents of N and P were similar to that of NK. Therefore, the unbalanced nutrients in soil from the improper input of inorganic nutrients accounted for the low yield in NK. In addition, using data from fourteen long-term fertilization experiments, Edmeades (2003)

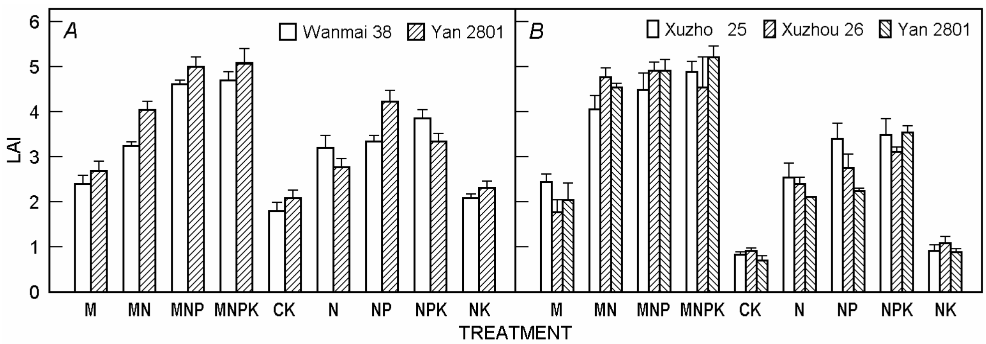

Fig. 5. Effects of long term fertilization treatments on LAI of different wheat cultivars at 20 d after anthesis (DPA) in the 2000–2001 season (*A*) and at 23 DPA in the 2001–2002 season (*B*). Each column with a bar indicates mean + S.E. of two replications for N and NK, and of four replications for other treatments.

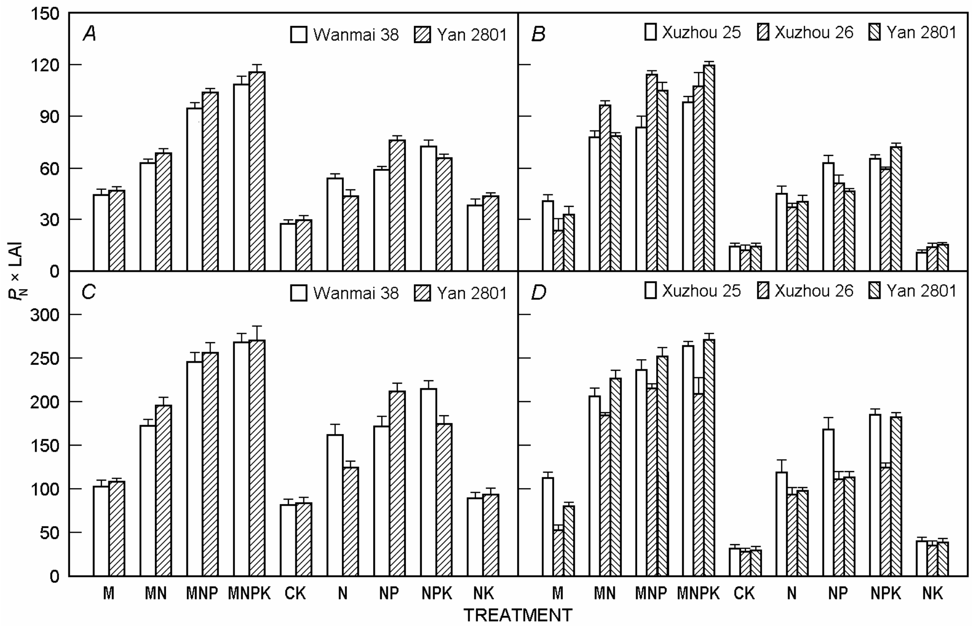

Fig. 6. Effects of long term fertilization treatments on P_N×LAI (*A, B*) and SPAD×LAI (*C, D*) of different wheat cultivars in the 2000–2001 (*A, C*) and 2001–2002 (*B, D*) seasons. Each column with a bar indicates mean + S.E. of two replications for N and NK, and of four replications for other treatments.

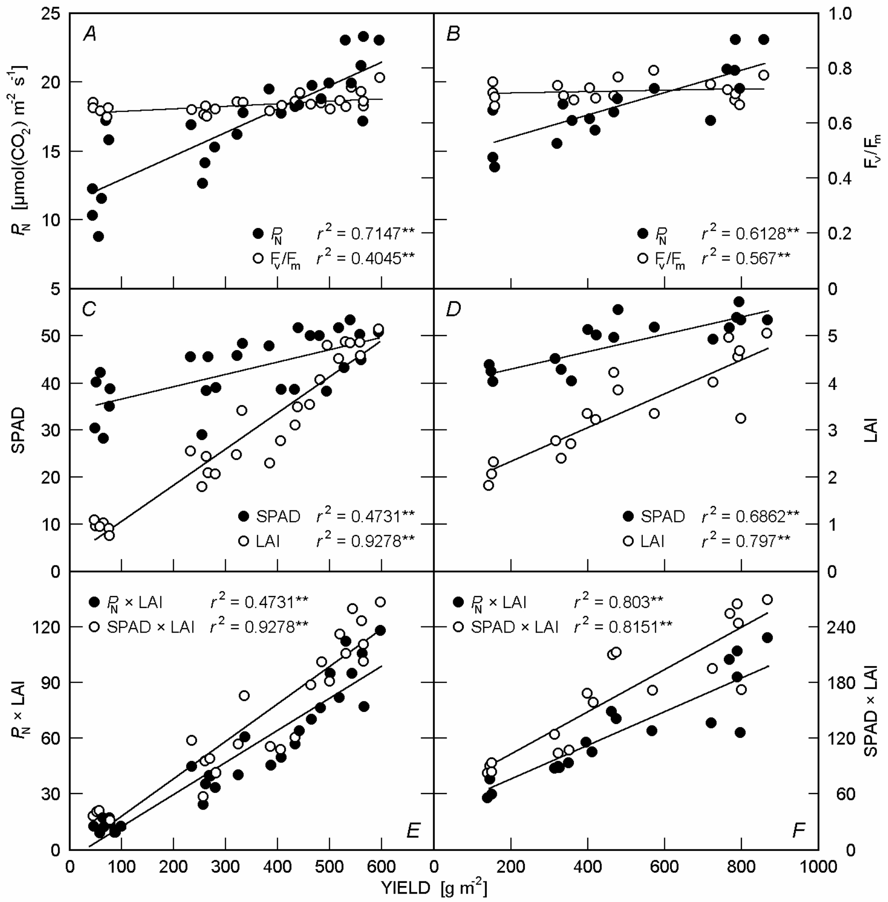

Fig. 7. Correlation of grain yield to P_N (*A, B*), F_v/F_m (*A, B*), and SPAD and LAI values (*C, D*) of flag leaf, P_N×LAI and SPAD×LAI (*E, F*) in wheat in the 2000–2001 (*A, C, E*) and 2001–2002 (*B, D, F*) seasons.**significant at 0.01 level.

concluded that yields with treatments of only organic manure applied were similar to that of only inorganic (mainly N) fertilizer. In the present study, however, yield with treatment of only M was lower than that of only N, and the combined organic and inorganic fertilization promoted grain yield in wheat. Therefore, the combined application of organic and inorganic fertilizers is of significant importance in wheat production, at least in the experiment area under study.

The photosynthetic capacity of leaf and LAI are key factors to determine wheat yield. P_N directly indicates the photosynthetic capacity of single leaf, F_v/F_m reflects the maximum photochemical capacity of PS2 in leaf (Arausa *et al*. 1998), and SPAD is the greenness of leaf, and represents the content of photosynthetic pigment (Rajcana *et al*. 1999, Ommen *et al*. 1999). As a result, these three parameters showed significant relationship to wheat yield (Fig. 7). As the canopy photosynthetic area, LAI was closely related to grain yield, with coefficients of 0.928^{**} in 2001–2002 or 0.797^{**} in 2000–2001. At the mid grain filling stage, P_N, F_v/F_m, and SPAD of flag leaf as well as LAI were higher and decreased more slowly, which could partly contribute to the higher yield in TMI, compared with TI. In addition, P_N×LAI and SPAD×LAI, the parameters for canopy photosynthetic capacity in wheat, showed close relationship to grain yield, with coefficients of 0.803^{**} to 0.889^{**}, indicating that the canopy photosynthetic capacity was much important for wheat yield than single leaf photosynthetic capacity. LAI, P_N×LAI, and SPAD×LAI were much higher in treatments of TMI than those in TI at the mid grain filling stage. Therefore, the improved canopy photosynthetic capacity could be another important contributor to the higher grain yields under combined application of organic and inorganic fertilizers in wheat. In treatment of NK, of course, the decreased photosynthetic capacities of both single leaf (expressed as P_N, F_v/F_m, and SPAD) and canopy (expressed as LAI, P_N×LAI, and SPAD×LAI) were the major reasons for the very low yield obtained with high input of N and K fertilizers.

Nitrate-N in soil increases with long-term application of organic manure (Powlson *et al.* 1989). When N was applied in organic form, N uptake in plants did not improve due to the large loss of nitrate-N (Johnston 1997). Edmeades (2003) also concluded that enrichment of N and P in soil occurred when the input of N and P *via* organic manure exceeded the need of crop, thus led to great N and P loss *via* runoff and leaching in soil. As a result, soil quality did not improve with long-term input of organic manure, compared with inorganic fertilizers. Under combined application of organic and inorganic fertilizers in the present study, there was also obvious enrichment of N and P in soil (Table 1), yet it was not clear if the N and P were lost *via* runoff and leaching in soil. Since the combined application of organic and inorganic fertilizers obviously increased grain yield in wheat, it should also increase the demand (or uptake) of N and P in wheat plants. Therefore, the combined application of organic and inorganic fertilizers could, at least partly, compensate for the probable loss of N and P in soil.

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