Grain Nitrogen Concentration in Wheat Grown under Intensive Organic Manure Application on Andosols in Central Japan

Yoichiro Kato

(Institute for Sustainable Agro-ecosystem Services (ISAS), The University of Tokyo, 1-1-1 Midoricho, Nishitokyo 188-0002, Japan)

Abstract: Grain nitrogen concentration (N%) is a major determinant of grain quality in winter wheat. The objective of this study was to compare the responses of wheat grain N% to organic manure with those to inorganic fertilizer in long-term experiments. We analyzed the grain N accumulation using soft wheat (Kinunonami) and hard wheat (Yumeshihou) cultivars grown with a high rate of organic manure application (OM; 80 t ha⁻¹ yr⁻¹ for >10 years and 30 t ha⁻¹ yr⁻¹ during the three years of the present study) and with standard (SF; 204–252 kg N ha⁻¹ yr⁻¹) or low (LF; 102–126 kg N ha⁻¹ yr⁻¹) rates of inorganic fertilizer for three years in Japan. The results agreed with previous research on the underlying mechanisms for grain N% under conventional fertilizer management: both sink and source regulation affected N accumulation in grains, and the accumulation of N in grains and of dry matter in grains are independent. Grain N% was significantly higher in the OM treatment than in the SF and LF treatments as a result of lower dry matter accumulation in the grains. High straw N% led to higher N accumulation in grains in the OM treatment during the late grain-filling period in Yumeshihou. Our results suggest that too much organic manure was applied, i.e., more than was required to optimize grain N%, when manure application was designed to produce a grain yield equivalent to that in conventional fertilizer management. We discuss ways to stabilize grain N% under intensive organic manure application.

Key words: Fertilizer management, Long-term experiment, Organic manure, Triticum aestivum.

Studies on fertilizer management regimes in winter wheat (Triticum aestivum) production have focused on achieving both high grain quality and high quantity (yield). Grain protein content, which is derived from grain nitrogen concentration (N%), is a major determinant of grain quality in wheat. For example, in Japan, the soft wheat used to make noodles and the hard wheat used to make bread require a grain N% of 1.9 to 2.3% and 2.3 to 2.8%, respectively (Nakano et al., 2010). However, grain N% is strongly affected by soil N availability, and a complex interaction between grain N% and grain yield exists (Triboi and Triboi-Blondel, 2002). The studies on the effects of the timing and amount of N topdressing on wheat yield and grain N% (Nakano and Morita, 2009; Nakano et al., 2010) have revealed that the optimal fertilizer management regimes for increasing grain N% and grain yield differ markedly. Accordingly, judicious N management (i.e., synchronizing N supply with plant demand) is necessary to optimize grain N% at a high yield level stably in conventional fertilizer management.

In addition to the need to optimize wheat productivity and grain value based on the intended use of the grain, there is an increasing demand for more sustainable resource use in intensive modern agriculture (Tillman et al., 2002). Replacing costly inorganic fertilizer with organic manure derived from readily available animal wastes to supply N to crops is one approach to pursuing more efficient nutrient cycling. For example, only 65% of livestock excrement (based on N content) is returned to agricultural land in Japan (Mishima et al., 2006). One reason for this may be that most experiments with organic farming systems have produced significantly lower wheat yields than conventional systems (Dawson et al., 2008). However, a high yield equivalent to that obtained using conventional fertilizer management was achieved in a wheat cropping system that relied on organic amendments as the sole source of N when the manure application rate was increased to 80 t ha⁻¹ yr⁻¹ (Kato and Yamagishi, 2011). Although such a high input of manure might have adverse environmental impacts, intensive wheat cultivation based on fertilization with organic manure may be possible if the N supply is balanced with crop needs to attain both high
In the LF treatment, half of the fertilizer amount used in 120–78–134 to 168–109–187 kg ha$^{-1}$ were 84–55–94 kg ha$^{-1}$.

grain N%.

understand the factors responsible for the variation in experiments conducted in central Japan. In particular, we with those to inorganic fertilizer regimes in long-term response of grain N% in winter wheat to organic manure of grain N% to changes in sink–source relationships should be analyzed separately to determine the responses dynamics of N and dry matter accumulation in grains independent (Foulkes et al., 2009). Accordingly, the grains and of dry matter in grains are relatively N top-dressing, and showed that the accumulation of N in grains and of dry matter in grains are relatively independent (Foulkes et al., 2009). Accordingly, the dynamics of N and dry matter accumulation in grains should be analyzed separately to determine the responses of grain N% to changes in sink–source relationships (Mattice et al., 2009).

The objective of the present study was to compare the response of grain N% in winter wheat to organic manure with those to inorganic fertilizer regimes in long-term experiments conducted in central Japan. In particular, we analyzed the process of N accumulation in grains to better understand the factors responsible for the variation in grain N%.

Materials and Methods

1. Field management

This study was conducted as a part of the long-term experiments started in 1993 as a part of the Institute for Sustainable Agro-ecosystem Services, The University of Tokyo, Japan (35°43′N, 139°32′E). The study was designed to compare fertilization systems that differed mainly in the type (organic manure vs. inorganic fertilizer) and intensity of fertilization in upland cultivation systems. The soil at the study site is a volcanic ash that is classified as a Typic Melanudand (USDA Soil Taxonomy). In the summer of 1993, three trials were established ($n=4$ plots per trial): standard input of inorganic fertilizer (SF), low input of inorganic fertilizer (LF), and high input of organic fertilizer (composted cattle manure; OM). In each plot, maize was grown from June to October, and wheat was grown from November to June. In the SF and LF treatments, compound inorganic fertilizer was applied before sowing of maize and again before sowing of wheat; the fertilizer was also side-dressed during maize cropping in the SF treatment. The N–P–K application rates in the SF treatment were 84–55–94 kg ha$^{-1}$ for winter wheat and 120–78–134 to 168–109–187 kg ha$^{-1}$ for maize in summer. In the LF treatment, half of the fertilizer amount used in the SF treatment was applied. In the OM treatment, composted cattle manure was applied in June of each year at a rate of 80 t ha$^{-1}$ until 2007, and at a rate of 30 t ha$^{-1}$ thereafter; these corresponded to N application rates of 739 and 277 kg N ha$^{-1}$, respectively. Aboveground plant residues were removed from the field between crops. In this paper, we analyzed data obtained from 2007 to 2009, after more than 10 years of the three fertilizer treatments. Wheat seeds were drill-planted in rows separated by 18 cm at a seeding rate of 80 kg ha$^{-1}$. We planted one soft wheat cultivar (used for noodle-making), ‘Kinunonamari’, and one hard wheat cultivar (used for bread-making), ‘Yumeshihou’. During the three years of our study, the 2007–2008 season was the coldest, and solar radiation from booting to early ripening (April) was highest in the 2008–2009 season. Although all crops were rainfed, there was no water stress because rainfall was adequate in all three seasons. Further details of the field management, including the set-up of the long-term trials and biomass production data, are described elsewhere (Kato and Yamagishi, 2011).

2. Measurements

At the double-ridge stage, the 50% heading stage, and 20 days after heading (DAH), two 1-m rows (0.35 m) were harvested from each plot; 30 to 50 stems were randomly selected as a subsample and were separated into stems, leaves, and spikes (if any). At physiological maturity (49 to 50 DAH), six 1.5-m rows (1.58 m$^2$) were harvested from each plot. We separated the spikes from the stems, and determined the grain weight and number after threshing. All samples were oven-dried at 80°C for at least 72 hours to determine their dry weights. The dried subsamples were ground, and the N concentrations were analyzed with an NC analyzer (Sumigraph NC-90A, Sumika Chemical Analysis Service, Tokyo, Japan). The N harvest index (NHI) was calculated as the N accumulation in the grains / total N accumulation. The N increases in the spike per grain from heading to 20 DAH and from 20 DAH to maturity (ΔN1 and ΔN2, respectively) were calculated as (spike N content at 20 DAH – spike N content at heading) / grain number m$^{-2}$ (determined at maturity) and (spike N content at maturity – spike N content at 20 DAH) / grain number m$^{-2}$, respectively.

We also collected 20 spikes from the main stems or the primary tillers at maturity to determine the variation in the grain weight and grain N% within a spike. Each spike contained 18 to 22 spikelets, and each spikelet contained 2 to 4 grains. We divided the spikelets in a spike into two portions: the lower part (first to tenth spikelet from the neck) and the upper part (spikelets starting with the eleventh spikelet from the neck). Grains in each spikelet were subdivided into the proximal (first and second florets) and distal (third and subsequent florets). We then gathered grains of the proximal florets of the lower
spikelets (LP grains), those of the proximal florets of the upper spikelets (UP grains), and those of the distal florets of the spikelets (D grains). We then determined the numbers, dry weights, and N concentrations (N%) in these divided samples.

The effects of fertilizer management were assessed by means of analysis of variance (ANOVA) for each variety (SAS Institute, 2003). Differences between means were compared using Duncan’s multiple-range test ($P = 0.05$).

Results

The mean yield across the three years in OM was not significantly different from that in SF treatment in Kinunonami (744 vs. 767 g m$^{-2}$), whereas the yield of Yumeshihou was significantly higher in the OM treatment than in the SF treatment (831 vs. 694 g m$^{-2}$) (Kato and Yamagishi, 2011). Differences between the treatments in the aboveground N corresponded to the differences in the annual total N input: in the OM treatment, total aboveground N was 53 to 87% higher than in the SF treatment, and 140 to 289% higher than in the LF treatment (Table 1). In the OM treatment, grain N% was significantly higher, but NHI was significantly lower than in the SF and LF treatments. There was no significant difference in NHI between the SF and LF treatments. Individual-grain N accumulation (mg grain$^{-1}$) tended to be lower in the OM treatment than in the SF treatment in Kinunonami, but the difference was only significant in 2008; there was no significant difference between the OM and SF treatments in Yumeshihou. The time course of aboveground N accumulation showed that wheat accumulated mostly during the reproductive stage, irrespective of the cultivar and fertilizer management treatment (Fig. 1). N accumulation during the post-anthesis period was small or negligible, indicating that the N required to support grain growth was mostly stored in vegetative organs and then it was remobilized and translocated to the grains.

![Image](https://example.com/image.png)

**Fig. 2** shows the time course of N% in the wheat straw (stem plus leaves) and in the spikes during the grain-filling period. N% in the straw at heading varied considerably with the fertilizer management treatment and the year. In the SF and LF treatments, N% in the straw did not vary with the year and cultivar at maturity although it greatly varied at heading. On the other hand, it varied greatly with the cultivar in the OM treatment: N% in the straw declined more sharply after 20 DAH in Yumeshihou than in Kinunonami. The change in N% in the straw varied little with the fertilizer regime from heading to 20 DAH, but varied more drastically in the OM treatment than in the SF and LF treatments from 20 DAH to maturity. Compared with N% in the straw, the variation and change in N% in the spikes was smaller and more gradual. The difference in N% in the spikes between fertilizer regimes was obvious at

| Cultivar, year | Management | Yield (g m$^{-2}$) | Biomass (g m$^{-2}$) | Total N (g m$^{-2}$) | NHI | Grain N% | Grain N (mg grain$^{-1}$) |
|---------------|------------|-------------------|---------------------|---------------------|-----|----------|---------------------------|
| Kinunonami, 2009 | OM | 862 a | 1920 a | 29.8 a | 0.51 b | 2.05 a | 0.50 |
| | SF | 853 a | 1612 b | 17.9 b | 0.74 a | 1.75 b | 0.54 |
| | LF | 449 b | 865 c | 8.6 c | 0.76 a | 1.65 b | 0.54 ns |
| Kinunonami, 2008 | OM | 664 a | 1437 a | 25.1 a | 0.52 b | 2.25 a | 0.56 b |
| | SF | 674 a | 1271 a | 15.4 b | 0.76 a | 1.97 b | 0.67 a |
| | LF | 395 b | 833 b | 8.7 c | 0.76 a | 1.88 b | 0.63 ab |
| Kinunonami, 2007 | OM | 707 | 1725 a | 25.2 a | 0.57 b | 2.30 a | 0.48 |
| | SF | 775 | 1424 b | 14.6 b | 0.76 a | 1.62 b | 0.51 |
| | LF | 661 ns | 1245 b | 10.5 c | 0.84 a | 1.51 b | 0.54 ns |
| Yumeshihou, 2009 | OM | 885 a | 1926 a | 31.1 a | 0.64 b | 2.54 a | 0.70 |
| | SF | 725 b | 1487 b | 16.6 b | 0.79 a | 2.05 b | 0.67 |
| | LF | 345 c | 760 c | 8.0 c | 0.82 a | 2.17 b | 0.70 ns |
| Yumeshihou, 2008 | OM | 777 a | 1506 a | 27.0 a | 0.69 b | 2.75 a | 0.81 a |
| | SF | 662 a | 1319 a | 17.7 b | 0.80 a | 2.45 b | 0.83 a |
| | LF | 377 b | 812 b | 8.9 c | 0.82 a | 2.18 b | 0.72 b |

In each section, means followed by the same letter within columns are not significantly different ($P < 0.05$) by DMRT. Data on grain yield and biomass were quoted from Kato and Yamagishi (2011).
heading, but the difference became minimal as the spikes grew (1.5 to 2.0% at 20 DAH). The difference in N% in the spikes increased again from 20 DAH to maturity, but the magnitude of the increase depended on the year and the cultivar.

The N increase per grain in the spikes during the early ripening period (heading to 20 DAH) tended to be lower in the OM treatment than in the SF and LF treatments, but the difference was significant only in Yumeshihou in 2008 (Table 2). We observed a similar trend in the increase in spike dry matter per grain, but the difference was significant only in Kinunonami in 2009. However, during the later ripening period (20 DAH to maturity), the N increase per grain was significantly higher in the OM treatment in Yumeshihou. The N increase per grain did not vary with the fertilizer regime in Kinunonami during either period.

The N increase per grain in the spikes was closely and significantly associated with the increase in spike dry matter per grain during the early ripening period \( (r=0.92 \text{ to } 0.95, P<0.01; \text{ Fig. } 3\text{a}) \). This relationship was similar in the two cultivars, indicating that grain N accumulation was coordinated with dry matter accumulation in the grains irrespective of cultivar and fertilizer regime. N increase per grain was not significantly correlated with either mean N% in the vegetative organs (i.e., the main source of N for grains) \( (r=0.83, P<0.05) \) with the N increase per grain in Yumeshihou, but not in Kinunonami \( (r=0.32) \).

Grain N% varied with the spikelet and floret within a spikelet (Fig. 4). In general, N% was the lowest in the D grains (distal florets within a spikelet) and the highest in the LP grains (proximal florets within a spikelet, which are located at a lower position in the spike) in all fertilization regimes and both years. Grain weight and grain N were always higher in the LP grains than in the UP grains (data
Table 2. Increases in spike N content per grain and spike dry weight per grain from heading to 20 DAH ($\Delta N_1$ and $\Delta W_1$), and those from 20 DAH to maturity ($\Delta N_2$ and $\Delta W_2$) in two wheat cultivars grown under different fertilization regimes.

| Cultivar, year       | Management | $\Delta N_1$ (mg grain$^{-1}$) | $\Delta W_1$ | $\Delta N_2$ (mg grain$^{-1}$) | $\Delta W_2$ |
|----------------------|------------|--------------------------------|--------------|--------------------------------|--------------|
| Kinunonami, 2009     | OM         | 0.11                           | 7.1 b        | 0.31                           | 18.8         |
|                      | SF         | 0.10                           | 7.9 ab       | 0.32                           | 22.7         |
|                      | LF         | 0.14 ns                        | 13.3 a       | 0.28 ns                        | 19.2 ns      |
| Kinunonami, 2008     | OM         | 0.14                           | 7.7          | 0.35                           | 16.5 b       |
|                      | SF         | 0.16                           | 10.7         | 0.42                           | 23.5 a       |
|                      | LF         | 0.15 ns                        | 11.6 ns      | 0.33 ns                        | 17.9 ab      |
| Yumeshihou, 2009     | OM         | 0.10                           | 10.5         | 0.49 a                         | 21.3         |
|                      | SF         | 0.13                           | 12.1         | 0.46 b                         | 22.0         |
|                      | LF         | 0.17 ns                        | 13.5 ns      | 0.41 b                         | 21.1 ns      |
| Yumeshihou, 2008     | OM         | 0.12 b                         | 10.3         | 0.50 a                         | 21.5         |
|                      | SF         | 0.19 a                         | 12.7         | 0.54 a                         | 23.3         |
|                      | LF         | 0.17 a                         | 13.0 ns      | 0.45 b                         | 20.2 ns      |

In each section, means followed by the same letter within columns are not significantly different ($P<0.05$) by DMRT.

Fig. 3. Relationships between the increases in spike N content per grain and (a) spike dry weight per grain and (b) mean N% in the straw (stem plus leaves) from heading to 20 days after heading. Relationships between the increases in spike N content per grain and (c) spike dry weight per grain and (d) mean N% in the straw from 20 days after heading to maturity. KN, Kinunonami; YS, Yumeshihou. Significance: *$P<0.05$; **$P<0.01$. Fig. 4. Grain N concentration (N%) in grains of the proximal florets of the lower spikelets (LP grains), grains of the proximal florets of the upper spikelets (UP grains), and grains of the distal florets of the spikelets (D grains). Range bars represent 2x the standard error of the mean (n=4). KN, Kinunonami; YS, Yumeshihou. Numbers after the cultivar indicate the year (08, 2008; 09, 2009).
the present study (Table 1). We suggest that the appropriate
the case in the soft wheat cultivar (Kinunonami) used in
ha
amount of soil N mineralization after manure application
Grain Nitrogen Concentration in Wheat in Japan
Grain Nitrogen Concentration in Wheat in Japan
not shown). Interestingly, the difference in grain N% between the LF, UP, and D grains was the largest in the OM treatment, and there was no difference in N% between these grains in the LF treatment, indicating that the variation in grain N% within a spike depends on the difference in the N supply available to support grain growth.

Discussion

A comprehensive literature review suggested that the timing of N supply from the soil rather than the total N supply was a problem for wheat production supported by the use of organic fertilizer (Berry et al., 2002). For example, the total N input for organically grown crops is estimated to be similar to that for conventionally managed crops in the U.K., with application rates of 150 to 300 kg N ha
1. However, either grain N% or grain yield in organically grown wheat is often lower than the standard level obtained with inorganic fertilizers (Baackstrom et al., 2004; Berry et al., 2002). In our long-term experiment, the high input of manure (80 t ha
1 year
1 during the study period and 30 t ha
1 year
1 during our study period) gave a grain yield equivalent to that in the standard fertilizer treatment (Kato and Yamagishi, 2011). In upland crop rotation systems in central Japan, such as the one in the present study, the N accumulation in winter wheat is greatest during the reproductive stage from late winter to early spring (Fig. 1). N uptake and crop growth during this period determine the total grain number (Fischer, 2008). Thus, the soil N supply in a treatment with a moderate rate of manure application may not fully support the plant’s N demand, and may therefore fail to produce a high grain yield, especially in a cold year (i.e., when mineralization of the organic N is slower).

On the other hand, a stagnant response in grain yield to increasing N supply is often associated with an increase in grain N% (Asseng et al., 2002). This means that researchers and farmers tend to apply more organic manure than is needed to produce optimal grain N% in order to ensure a high grain yield in organic culture, as was the case in the soft wheat cultivar (Kinunonami) used in the present study (Table 1). We suggest that the appropriate application rate of organic manure required to achieve both optimal grain N% and high grain productivity should be carefully determined by simulating the pattern and amount of soil N mineralization after manure application in a given soil type and under a given temperature regime.

The physiological mechanisms responsible for the regulation of grain N% have been extensively studied in wheat (Triboi and Triboi-Blondel, 2002). The grain N content is considered to be regulated both by sink, i.e., the plant’s intrinsic capacity to translocate N into the grains, and source, i.e., the N supply available for translocation to the grains. The results of our study agreed with these previous results. The similar rate of decrease in straw N% in all cultivars and fertilizer regimes from heading to 20 DAH and the relatively constant spike N% after 20 DAH were unaffected by these changes in straw N% (Fig. 2), indicating that N accumulation in the grains is largely regulated by the sink, not the source, during the early grain-filling period. This is also supported by the fact that N accumulation in each grain was coordinated with dry matter accumulation in the spikes and was unaffected by straw N% (Figs. 3a, b). In contrast, the magnitude of the changes in straw and spike N% and in N accumulation per grain were affected by straw N% during the late grain-filling period (Fig. 3d), though the extent depended on the cultivar. Our observations confirm those of a modeling study that described the N dynamics during the grain-filling stage in wheat (Martre et al., 2003): N accumulation in the grains was mostly regulated by the sink strength until 15 days after anthesis, i.e., about 20 DAH, and mostly by the source strength from 15 days after anthesis to maturity. These phenomena can be explained by the functional role of accumulated N during each stage of grain protein synthesis: N is accumulated in the grains as structural and metabolic proteins (albumins, globulins, and amphiphilic proteins) during the early stages of grain growth, and as storage proteins (gliadins and glutelins) thereafter (Triboi et al., 2003).

Another widely accepted physiological hypothesis for the determination of grain N% is that N accumulation in the grains is independent of dry matter accumulation (Asseng et al., 2002; Triboi and Triboi-Blondel, 2002). We also observed this phenomenon in the present study: we saw a large difference in grain N% as a function of the position within a spike, and the difference was clearer the higher the N availability (Fig. 4). It is well-known that grain N% in wheat is higher in the grains in spikelets at lower to middle positions in the spike, and in the grains at basal (proximal) positions in a spikelet (Bremner and Rawson, 1978; Herzog and Stamp, 1983). If the accumulations of N and dry matter in grains were co-regulated at a predetermined ratio, the grain N% would not vary within the spike, nor would the variation in grain N% respond to changes in N availability.

Organic fertilizer management for high yield requires genetic and agronomic improvements to stabilize grain N%, since this approach can lead to supraoptimal grain N%. The grain N% of organically grown wheat could be maintained at the optimal level in two ways: by controlling N inflow into the grains and by intensifying post-anthesis photosynthesis. The capacity for N accumulation in the grains during the late grain-filling period varied with the cultivar: N accumulation in the grains responded more strongly to the source capacity in Yumeshihou (hard wheat) than in Kinunonami (soft wheat) (Fig. 3d; Table 1). In vitro experiments that controlled the N supply to the
grains showed that genotypic differences in grain N% were derived, at least in part, from differences in the capacity for protein synthesis (Wyss et al., 1991). In the N-enriched soils caused by high rates of manure application, it would be desirable to down-regulate the synthesis of storage proteins in soft wheat cultivars. As the grain N% is not strongly affected by modification of the protein composition (Rooke et al., 1999; Stone and Nicola, 1996), it is worthwhile modifying the capacity for synthesis of storage proteins so that this process is independent of N availability. Alternatively, it would be possible to control N accumulation in the grains by modifying the activity of the enzymes that are responsible for N remobilization, such as the glutamine synthetase–glutamate synthase enzyme system (Kichey et al., 2007). Although there have been some examples of source-regulated gene expression for synthesis of storage proteins (Uauy et al., 2006), functional genomics and molecular physiology studies of grain protein synthesis and N metabolism will expedite attempts to fine-tune grain N% in the future (Dupont and Altenbach, 2003).

An alternative approach concerns the improvement of dry matter accumulation in grains. Despite some differences in grain N content between fertilizer regimes, differences in grain N content could not fully explain the much higher grain N% in the OM treatment than in the SF and LF treatments (Table 1). Grain N content (mg grain⁻¹) was lower in the OM treatment than in the SF and LF treatments in Kinunonami, which is the opposite of the results for grain N%. This was largely due to a lighter grain weight in the OM treatment (Kato and Yamagishi, 2011). Since dry matter accumulation progresses independently of N accumulation in the grains, increasing grain weight by enhancing concurrent photosynthesis may not significantly change the N partitioning to the grains (i.e., NHI), and may contribute to the stabilization of grain N% under high N supply levels from the vegetative organs. Since our previous results showed that post-anthesis crop growth was also relevant to the harvest index and grain weight under high manure application rates (Kato and Yamagishi, 2011), post-anthesis growth would be the key growth characteristic required to produce high grain yield and high grain quality in winter wheat cultivated within an intensive organic cropping system.

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