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## REVIEW
# Effects of Fertilizer and Organic Amendments on Metabolite Profiles in Radish, Komatsuna, and Mizuna

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### Abstract
Mechanisms underlying the effects of organic amendments on the metabolites in vegetable crop plants remain unclear for the most part. Organic fertilizers are believed to help produce "high quality" vegetables; however, the relationships between organic amendment and vegetable quality are not understood. We took a metabolite profiling approach that comprehensively analyzed both the major and minor constituents of plants. Manure amendments consistently affected the metabolite composition of various vegetable organs in field experiments. Moreover, it was shown that both manure and organic fertilizer amendment caused clear metabolic changes in pot experiments using mizuna (*Brassica rapa* L. var. Nipponsinica). It is suggested that the amendment of organic matter does indeed influence metabolite composition in vegetables. Further research is needed on the mechanisms responsible for changes in metabolites and on the factors affecting the quality characteristics of foods.

**Discipline:** Soils, fertilizers, and plant nutrition
**Additional key words:** amendments of organic matter, GC/MS, metabolite profiling, vegetables, water soluble components

## Introduction

Vegetables grown organically or using organic fertilizer(s) are often claimed to be high quality foods, or having favorable taste. However, there had been little scientific evidence supporting the perceived quality of organic foods (Bourn and Prescott 2002, Brandt and Molgaard 2001, Hajslova et al. 2005, Lima and Vianello 2011, Woese et al. 1997). It is in our interest to know whether organic vegetable plants differ scientifically from non-organics. Ordinary approaches now being taken to evaluate the quality of agricultural products are based on several certain constituents reportedly related to 'good' taste or involved in human health. Similar approaches have been adopted relative to organic foods, in which limited specific compounds speculated as unique constituents are often analyzed. Those approaches often ignore certain trace and/or minor metabolite constituents, despite the possibility of them having unknown effects or interactions with other constituents. Recently, the "metabolomics" approach has been applied in the area of food quality control as discriminative, predictive, and informative methods (Cevallos-Cevallos et al. 2009, Varela and Ares 2012). Food properties are highly influenced by such constituents as sugar, organic acids, and amino acids — the targets of metabolomics (Azodanlou et al. 2003, Malundo et al. 1995). The metabolite profiling approach, entailing a comprehensive analysis of food constituents, is expected to become a powerful tool to evaluate the quality of organics. Gas chromatography and mass spectrometry (GC-MS)-based metabolite profiling, involved in the analysis of sugars, organic acids, and amino acids, has been widely employed as an efficient and convenient tool for the metabolomics approach (Ribeiro et al. 2009, Ricroch et al. 2011, Shepherd et al. 2014). The traits of organic vegetables have been explained mainly by nitrogen absorption. Organically grown vegetables often have lower nitrogen absorption than conventionally grown vegetables, thus leading to higher sugar and lower amino acid concentrations (Rembialkowska 2007). Dairy cattle manure is widely used by Japanese farmers as a soil improver with weak fertilizer effects, while fast-release organic fertilizer is often used as an alternative to chemical fertilizers in "special cultivation" or "organic farming" systems in Japan. In this review, we describe the results of metabolomics research on vegetable

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crop plants applied with organic materials, including dairy cattle manure and fast-release organic fertilizer, basically based on our experiments.

## Manure-induced metabolite changes

### 1. Effects of manure application on metabolite composition in radish

We investigated the effects of dairy cattle manure on radish metabolites in fields of the Hokkaido Agricultural Research Center (HARC) (Okazaki et al. 2010), applied with manure (0, 2, 4 kg $m^{-2}$) and inorganic nitrogen fertilizer ($N_{inorg}$). Sugars, organic acids, and amino acids in leaf and root were analyzed using GC-MS, followed by Principal Component Analysis (PCA) for the combined data from the peak area of metabolites. The scores for PC1 and PC2 corresponded to $N_{inorg}$ (accounting for 46.5% of the total variance) and manure (14.6% of the total variance), respectively (Fig. 1). It was also shown that leaf metabolites responded more strongly to both $N_{inorg}$ and manure application than did root metabolites. Amino acid concentrations clearly responded to $N_{inorg}$ application, but not to manure. The concentrations of malic acid, myo-inositol-6-phosphate, and sucrose decreased after the application of manure amendments, whereas concentrations of shikimic acid, arabinose, and L-methionine increased. This shows that the effects of manure amendments and nitrogen on radish appeared in the leaf metabolites. The effects on metabolite concentrations were observed as PC1 and PC2, and distinguished by PCA.

### 2 Effects of nitrogen, manure, and slow-release nitrogen fertilizer

It has been known that manure amendments increase the availabilities of nitrogen, phosphorus, and potassium (Clapp et al. 2007, Eghball 2002, Kansal et al. 1981, Sager 2007, Wen et al. 1997). To confirm these effects from the perspective of metabolomics, we investigated metabolite profiles using komatsuna (*Brassica rapa* L. cv. Minami) applied with manure, fast-release N (as ammonium sulfate), slow-release N (coated ammonium nitrate), and P and K fertilization (Okazaki et al. 2012). Metabolite profiling was conducted using GC-MS and PCA undertaken to test the effects of the amendments. Analysis of a total 129 metabolites in the leaves and petioles showed that the scores of PC1 (accounting for 44.1% of the total variance) and PC3 (8.8% of the total variance) were discriminated by N absorption and manure, respectively (Fig. 2). An analysis of variance (ANOVA) of the treatment effects on individual metabolites revealed the three most significant factors to be N absorption, manure, and slow-release N (44, 13 and 1 metabolite in leaf tissue, respectively; 45, 20 and 14 metabolites in petiole tissue, respectively). Slow-release N resulted in the slow absorption of N by the plants and higher concentrations of amino acids in the petiole. Manure and slowly absorbed N are thought to have the same effects

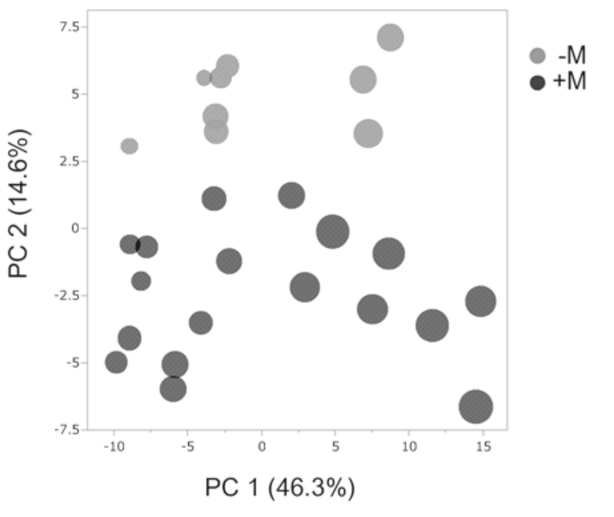


**Fig. 1. Sample scores for the first (PC1) and second (PC2) principal components drawn from principal component analysis of identified metabolites in radish leaf and root extracts. –M and +M denote the absence and presence of manure amendment, respectively. The size of each circle corresponds to the amount of nitrogen absorption. (Modified from Okazaki *et al*. 2010)**

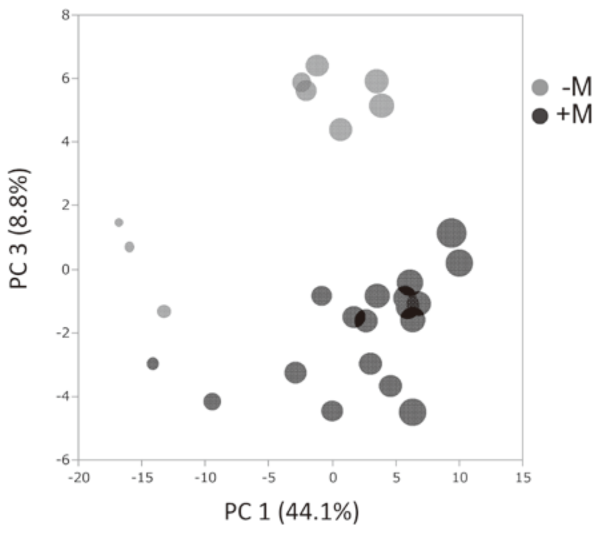


**Fig. 2. Sample scores for the first (PC1) and third (PC3) principal components drawn from principal component analysis of identified metabolites in komatsuna leaf and petiole extracts. –M and +M denote the absence and presence of manure amendment, respectively. The size of each circle corresponds to the amount of nitrogen absorption. (Modified from Okazaki *et al*. 2012)**

(Abbasi et al. 2007, Adegbidi et al. 2003, Hadas et al. 1983). If it is true, slow-release N could explain the effects of manure on metabolite compositions; furthermore, the pattern of significant metabolites by ANOVA should be similar between manure and slow-release N. However, the slow-release N and manure amendments showed different patterns, suggesting that they were metabolized differently. Chemical factors such as P or K did not discriminate PC1, PC2, and PC3. Several studies failed to indicate the relationships between metabolite levels and the ratios of organic matter amendments (Woese et al. 1997, Zorb et al. 2006). However, in the case of spinach, yield, P content, and ascorbic acid content were higher when applied with higher levels of nitrogen fertilizer and farmyard manure (Kansal et al. 1981). In our experiment, N, P, and K absorption were increased by manure amendments. The $P_2O_5$ and $K_2O$ inputs with 4 kg m$^{-2}$ manure amendments were 28 and 95 g m$^{-2}$, respectively, in which levels of P and K input exceeded the rate of inorganic $P_2O_5$ and $K_2O$ fertilization. We consider that the metabolite differences corresponding to PC3 were not explained by the N, P, or K nutrient status. Of the many metabolites in komatsuna, N was the only significant element affecting metabolite concentration as predicted by linear models. The results reveal that some effects of manure are considered as slowly absorbed N, but some are not.

## Organic fertilizer-induced metabolite changes: effects of manure and fast-release organic fertilizer application on metabolite composition in mizuna

Watanabe et al. (2013) investigated the effects of fast-release organic fertilizers and manure application on the metabolite composition of mizuna. It has been proposed that such fertilizers and manure contain different nutrients and affect the physical and biological properties of soil. Mizuna (*B. rapa* L. var. Nipponsinica) is Japanese leafy vegetable belonging to the Brassicaceae family and a popular foodstuff in Japan. Some consumers claim that mizuna produced by organic farming has a bitter and rich taste associated with a unique combination of metabolites due to the application of organic matter. To investigate the effects of fast-release organic fertilizers and manure, three treatments were employed separately: chemical (C), organic (O), and chemical plus manure (C + M) fertilization. The simple treatment of manure was not included due to difficulties in comparing it with the C or O treatments because of low N absorption. The O treatments were further divided into fish cake (Of), chicken droppings (Oc), and rape seed cake (Or). Sugars, organic acids, and amino acids were analyzed by GC-MS, followed by PCA using peak areas of a total 71 identified metabolites. PC1 accounted for 33.4% of the total variance and showed a significant correlation ($P < 0.01$) with N absorption. PC2 explained 15.6% of the total variance and discriminated between C and O treatments. PC4 accounted for 6.6% of the total variance and separated C and C+M treatments (Fig. 3). Clear discrimination between the treatments using PC2 and PC4 scores suggested that metabolite compositions were influenced by fertilizer origin (inorganic and fast-release organic fertilizers) or manure applications. The results indicated that the levels of manure amendments, nitrogen, and fast release organic fertilizers reflected in the leaf metabolites of mizuna..

Zorb et al. (2009) compared the metabolites of wheat between organic and conventional systems, and reported that the organic system showed a significant decrease in the content of 14 and 11 amino acids in wheat ears and grain, respectively. In pot experiments using spinach, the concentrations of some amino acids were low under the organic system as compared to the conventional (Takeda 2012). Higasa et al. (2012) investigated the differences in the constituents of spinach and komatsuna between organic and conventional farming. They reported that the concentrations of free amino acids in spinach plants grown under the organic system were significantly less than under the conventional system, whereas there was no difference in komatsuna. Thus, the concentration of amino acids in vegetable plants apparently decreases when organic fertilizers are applied. However, we cannot exclude the possibility that those responses are limited in certain species such as mizuna.

## Conclusion and future research

We investigated the effects of a cultivation factor focused on nutrient management and organic matter amendments based on the metabolite profiles of several vegetable species. In the leaves of plants of the brassica genus, the concentrations of sugar, organic acid, and amino acid are mainly influenced by N absorption. It was shown that manure amendment affects the metabolite compositions of all species. It was also observed that the degree of effects differed when the value was compared among the organs of each species. On the other hand, the application of phosphorus, potassium, and fast-release organic fertilizers had a relatively small contribution as compared to N fertilizer or manure. These results suggest a need to focus on the mechanisms of organic matter decomposition, which could contribute to the generation of minor elements due to interactions between soils and plant roots. Such experiments require deeper spatial scales and longer time spans, because such rhizosphere circumstances as soil structure, water availability, and microorganisms will interact with the decomposition of organic matter. Although certain effects of manure amendment and fast-release organic fertilizers on amino acid content were detected, their contribution to

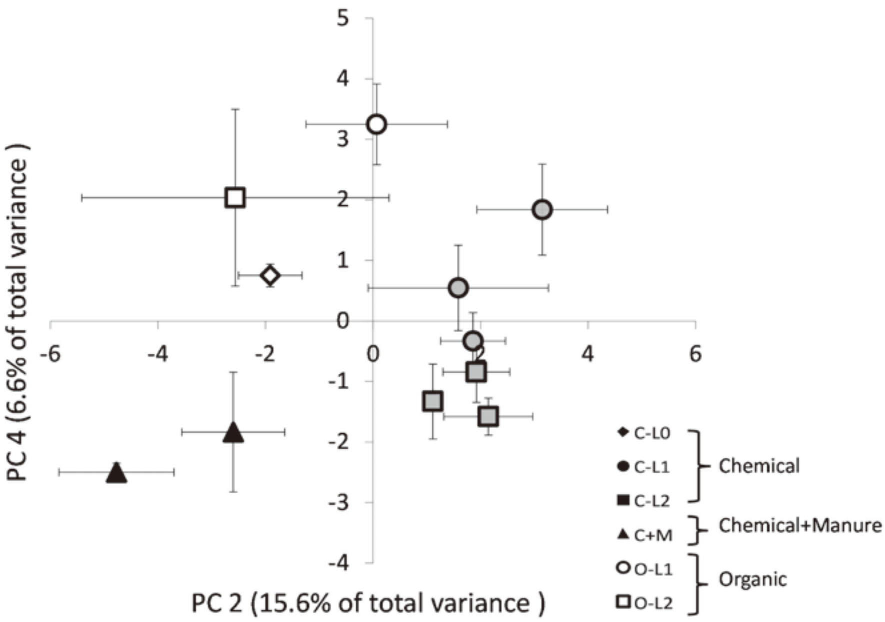


**Fig. 3. Principal component analysis (PCA) of metabolites in shoot extracts of mizuna. Scores of PCA are presented based on combinations of PC2 and PC4. Each group was calculated from four samples. Error bars represent standard error. (Modified from Watanabe *et al.* 2013)**

food quality remains unknown. In our study, metabolites varied with N absorption, manure amendment, and organic fertilizer application. However, significant correlations between these metabolites and quality have been previously reported (Bernillon et al. 2013, Tanaka et al. 2015, Ursem et al. 2008). In the future, further investigations will reveal the correlation between metabolite profiles and food quality characteristics in greater detail.

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