Amino acid and peptide dynamics in horticultural soils under conventional and organic management strategies

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Abstract

Purpose Free amino acids (FAAs) and peptides, and dissolved organic nitrogen (DON) comprise key pools in terrestrial soil carbon (C) and nitrogen (N) cycles. A comparative study of organic and conventional arable farming systems was conducted in Shanghai, China to determine the influence of management practices on characterization of AA and peptide dynamics.

Materials and methods Soils from an accredited organic farm (OS), transitional farm (TS), and neighboring conventionally managed farm (CS) were sampled from agricultural areas in Shanghai, China, and the AA and peptide behavior of top soils were evaluated by ^{14}C labeled techniques.

Results and discussion Results showed that organic management resulted in significant increases ($P<0.05$) in AA and peptide rate acquisition, possibly due to higher soil organic C (SOC) and soil respiration in OS, compared to TS and CS.

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The half-life of valine (Val), valine-proline-proline (Val-Pro-Pro), glutamate (Glu), and glutamate-phenylalanine (Glu-Phe) averaged across the three soils (OS, TS, and CS) was 10.6±2.3, 9.4±2.2, 7.1±1.9, and 6.7±1.5 h, respectively. The relative AA and peptide C amounts partitioned into respiration (15–32% of total) versus biomass production (68–85% of total C) was also affected by management strategy and generally followed the order CS>TS>OS; OS was significantly different from TS and CS; however, a significant difference was not detected between TS and CS. We hypothesize that AA and peptide mineralization acts as a purely biological process, as $CHCl_3$ fumigation and autoclaving resulted in no observable mineralization. Results showed that AA and peptide addition to soil resulted in rapid N mineralization and subsequent NO_3^-–N production, which followed a progression based on soil N mineralization rate of OS>TS>CS. AAs and peptides were only weakly sorbed onto the soil solid phase; however, our results suggested that this did not limit soil bioavailability.

Conclusions The results of our study indicated that soil management regimes have a substantial effect on AA and peptide behavior (mineralization, microbial uptake, and sorption, among other attributes) in horticultural production systems. Therefore, we propose that SOC and total microbial activity are likely key determinants governing AA and peptide behavior in horticultural soils.

1 Introduction

A large percentage of the Earth's active carbon (C) is deposited in soil organic matter (SOM), and its cycling rate is

tightly linked to nitrogen (N) availability in natural and managed ecosystems (Gärdenäs et al. 2011). SOM turnover, the formation of dissolved organic N (DON), and its subsequent turnover to inorganic N play a key role in soil fertility and available nutrient supply to both vegetation and soil microorganisms. Mengel (1996) reported that primary SOM contribution to soils includes approximately 5–25 of soil N, which provides free amino acids (FAAs), peptides, and proteins. FAA and combined AAs typically constitute approximately 10–20% of soil organic C, and 30–40% of soil N (Stevenson 1982). However, AAs are typically present mostly in a polymeric state (i.e., peptides and proteins), while total FAA concentrations are generally low, representing <0.06% of the total soil N (Ge et al. 2010a, b). The sorption, microbial uptake kinetics, and mineralization of FAA and free amino sugars in soils is well established (van Hees et al. 2005; Knowles et al. 2010); however, little is known regarding the short-term behavior of oligopeptides (e.g., di- and tri-peptides) in soil.

It is generally accepted that the associated effects of exogenous substrate including quality and quantity of microbial species, together with primary soil physical and chemical properties, regulate ecosystem biological structure and function (Yoo et al. 2006; Jones et al. 2009). Certain parameters including soil structure, temperature and water content will also greatly influence soil biological activity; consequently, C mineralization rate (Knorr et al. 2005; Yoo et al. 2006) and SOM (including FAAs, peptides, and proteins) turnover is closely regulated by the activity of by soil microorganisms. Therefore, to design sustainable agricultural systems and reduce N pollution, we must first gain a better understanding of the biological systems, functions, and responses to soil conditions. One functional aspect in soils is the utilization of FAAs and peptides.

FAAs, peptides, and proteins comprise the largest input of organic N (ON) in most soil environments. The majority of ON molecules enter the soil from root exudation and plant residues. The FAA and peptide inputs are normally accompanied by a variety of other labile C compounds including polysaccharides, simple sugars, amino sugars, and organic acids (Vaughan et al. 1993). Additional ON molecular components are derived from dry and wet deposition, rainfall, litter fall, animal residues (urine and feces), and soil organic fertilizer applications (Kalbitz et al. 2000). Following FAA and peptide release into soil habitats, these new soil constituents can undergo a number of possible fates including: (1) uptake by soil microbial biomass; (2) uptake by plant roots; (3) abiotic decomposition; (4) sorption to the soil solid phase; (5) formation of SOM (i.e., humic polymers, phenolics); (6) leaching into ground water; and (7) atmospheric CO_2 emission via respiration (Burkovski and Krmer 2002; Roberts et al. 2007; Tian et al. 2010). To date, most studies have applied unnaturally large FAA and amino

sugar concentrations to soil and (Roberts et al. 2007; Jones et al. 2009) the reports of peptide–soil interactions are limited.

Organic farming is gaining worldwide acceptance and has been expanding at an annual rate of 20% in the last decade, accounting for over 32.2 million ha worldwide (Willer and Lukas 2009). In China, 300,000 ha are currently farmed organically or are in the process of conversion. In Eastern China (e.g., Shanghai), organic vegetable production has intensified over the last few years to meet market demands (Ge et al. 2011). Organic fruit and vegetable production does not apply chemical fertilizers and pesticides, relies on organic input and recycling for nutrient supplies, and emphasizes cropping system designs and biological processes for pest management, as defined by world organic farming regulations such as the International Federation of Organic Agriculture Movements (IFOAM), the Japanese Agricultural Standards (JAS), and the European Union Standards. The influence of organic and conventional management practices on soil chemistry, microbial activity, and biomass has been extensively studied (García-Ruiz et al. 2009; Ge et al. 2011); however, few studies have focused on exogenous FAA and peptide utilization by soil microbial populations in horticultural production soils.

Based on the above considerations, the aim of this study was to clarify the effects of organic horticultural soil management strategies on the behavior of exogenously added FAAs [valine (Val), glutamate (Glu)] and peptides [glutamate-phenylalanine (Glu-Phe), valine-proline-proline (Val-Pro-Pro)] in organic and conventional management systems in Shanghai, Eastern China.

2 Materials and methods

2.1 Location and management

Different horticultural soil management strategies applied by Xia-Xi-Yang Organic Vegetable and Fruit Horticultural Farming, Jiading District, Shanghai, China (31°4′N, 121°24′ E) were tested in this study. The site is located approximately 2.5 km from the Yangtze River and the East China Sea. The study area occurs at an elevation 4 m above sea level; the climate is characterized as humid subtropical with 70% of the annual precipitation (1,255 mm) occurring between May and September; the mean annual air temperature is 17.5°C; and the annual average sunshine is 1,778 h (Ge et al. 2011).

The sampling sites were farmed using an organic, transitional, and conventional approach. The China Organic Farming Development Center (OFDC) accredited the organic and transitional horticultural methods. Consequently, chemical fertilizers, pesticides, or genetically modified

organisms were not used for at least 3 years. Reducing conditions were more notable in the organic portion of the trial, as the farm irrigation technique resulted in permanent water table depths between 0.4 and 0.7 m below the soil surface. The organic farm (approximate 20 ha) was certified (certification no. OF-3105/3106-931-243-2005) by the OFDC in June 2004 after a 2-year conversion period, the transitional farm (about 27 ha) was certified in June 2003 (certification no. V-3106-931-264-2003). The conventional plots (established for >6 years) were situated in a greenhouse close to the organic and transitional plots. Vegetable and non-tree fruit crops are typically grown in plastic tunnels. These fields had been conventionally cultivated for more than 6 years. The conventional fields were only occasionally irrigated. Farmers completed a questionnaire concerning farm practices for the last 3 years such as (cover) crops, amount and type of animal and green manure, and/or fertilizer used, pesticides, disinfectants, mechanical weeding, soil improvements, and plowing depth. Soils were evaluated in plastic tunnel fields under the following management practices: (1) organic (OS); (2) transitional (TS); and (3) conventional (CS). Details regarding the major crops grown and fertilizers used in the various fields are summarized in Table 1.

2.2 Soil samples

Soils were sampled during final harvest to avoid the effects of direct fertilization during the next growing season. For all (OS, TS, CS) soils, the Ah horizon was sampled in triplicate from a depth of 0–20 cm. Briefly, A stainless steel corer (5.5-cm diameter) was used to collect soil samples at each field from 10–15 randomly selected positions over a (10× 30 m) 300 m² area within each vegetable planting row. The soils were mixed to obtain three composite samples per field. Soil samples were immediately transported to the laboratory in gas-permeable plastic bags placed in ice-filled containers, and each sample was thoroughly mixed and sieved through a 2-mm mesh to remove plant matter and earthworms. One half of the fresh samples were stored at 4°C until analysis, and the remainder were dried in a

thin layer for 2 days at 30°C, and stored at room temperature in dark plastic bags. All soils were analyzed within 10 days of collection. Studies with similar soils has indicated that storage of samples for up to 2 months in this state (4°C) does not significantly affect their AA mineralization profiles (Jones et al. 2005). The soil was classified as a fluvisol developing toward a cambisol (according to FAO 1998), containing approximately 9.3% sand, 70.7% silt, and 20% clay to a soil depth of 40 cm. The main soil characteristics are presented in Tables 2 and 3.

2.3 Soil solution preparation

Soil solution was extracted by centrifugation drainage within 24 h of collection using the technique of Giesler and Lundström (1993). Approximately 100–120 g of field-moist soil was placed into a nylon centrifugal extraction cup with a perforated base. A lower vessel was placed underneath the cup containing the soil, and the device was centrifuged for 20 min at $8,000 \times g$. The soil solution was recovered from the lower vessel. Typically, 1–5 ml of soil solution was extracted from each soil sample, depending on the initial soil moisture content. The soil solutions were stored frozen in polyethylene vials at −20°C until analyzed.

2.4 Chemical analyses

Soil pH was determined in a 1:2.5 (w/v) soil/water suspension. Soil moisture was established gravimetrically by drying at 105°C to a constant weight. Total organic C (TOC) was determined using dichromate oxidation (Nelson and Sommers 1982); total N (TN) was measured with a CHN-2000 analyzer (Leco, St. Joseph, MI, USA); and exchangeable cation (K^+, Ca^{2+}, Mg^{2+}, and Na^+) concentration and cation exchange capacity (CEC) were measured using the titration method of Rhoades (1982). Soil respiration at a quasi-steady state was evaluated on 20 g of soil at 20°C using an automated multichannel SR1-IRGA soil respirometer (PP Systems, Hitchin, UK) immediately following soil field collection.

Table 1 Land management practices investigated in this study

Soil[a]	Present vegetation	Annual fertilizer input	Farming system	Conditions
OS	Tomatoes, Strawberries	Pig manure (40.0 tha⁻¹); organic fertilizer (2.8 tha⁻¹)	Organic farming	Plastic tunnel
TS	Cauliflower	Pig manure (40.0 tha⁻¹); organic fertilizer (2.8 tha⁻¹)	Transitional farming	
CS	Cantaloupe	Complex fertilizer. (N-P-K: 14-16-15; 350 kg ha⁻¹); Urea (250 kg ha⁻¹)	Conventional farming	

[a] Soils were evaluated in plastic tunnel fields under the following management practices: (1) organic (OS); (2) transitional (TS); and (3) conventional (CS)

Table 2 Soil chemical properties (0–20 cm depth) from three horticultural soils (OS, TS, CS)

Soils[a] (c mol kg⁻¹)	pH	SOC (g kg⁻¹)	Total N (g kg⁻¹)	C:N ratio	Moisture content (g kg⁻¹)	Soil respiration (μmol CO2 kg⁻¹ h⁻¹)	Exchangeable cations (c mol kg⁻¹)				CEC
							Ca^{2+}	Mg^{2+}	K$^+$	Na$^+$	
OS	7.2±0.1 ac	17.6±0.2a	1.5±0a	11.4±0.2c	198.8±9.5b	269.4±12.5a	16.07±0.32a	5.53±0.31a	1.13±0.07a	2.29±0.07a	21.36±0.36a
TS	7.4±0.1a	12.3±0.3b	0.73±0.05c	17.1±0.2a	171.9±7.5c	248.4±15.9a	14.65±0.55b	2.54±0.08b	0.29±0.04c	0.40±0.03c	13.66±0.38c
CS	7.7±0.1a	12.5±0.5b	0.91±0.07b	13.9±0.2b	222.8±2.2a	209.4±19.5b	14.55±0.27b	3.04±0.21b	0.63±0.03b	0.62±0.02b	16.02±0.18b

Values represent means±SEM (n=3). Means followed by different letters indicate significant differences at $P<0.05$ by Duncan's test procedures

CEC Cation exchange capacity.

[a] Soils were evaluated in plastic tunnel fields under the following management practices: (1) organic (OS); (2) transitional (TS); and (3) conventional (CS)

A Shimadzu TOC-TN analyzer (Shimadzu, Kyoto, Japan) was used to measure total dissolved N (TDN) in the soil solution as described by Jones et al. (2004). NO_3^- and NH_4^+ were determined using a Skalar segmented flow auto-analyzer (Skalar, Norcross, GA, USA) and the methods described in Mulvaney and Mulvaney (1996). DON was calculated as the difference between the TDN reading, and the sum of NO_3^- and NH_4^+. Total free AAs (FAAs) were established on a Varian Cary Eclipse 96-well plate fluorescence spectrophotometer (Varian, Palo Alto, CA, USA) following the *o*-phthialdehyde β-mercaptoethanol procedure of Jones et al. (2002). Protein was assessed with bovine serum albumin as a standard using the Bradford (1976) protocol. Phenolic concentrations were established using Folin-Ciocalteu reagent, calibrated with a phenol standard according to Swain and Hillis (1959). Soil solution color was determined at 630 nm with a Versamax microplate reader (Molecular Devices, Sunnyvale, CA, USA) to determine the humic substance concentration (Hill et al. 2006).

2.5 ^{14}C AAs and peptide properties

Two uniformly labeled AAs (^{14}C-labeled Val, Glu) and two uniformly labeled peptides (^{14}C-Glu-Phe, ^{14}C-Val-Pro-Pro) were chosen as compounds due to commercial availability; in addition, the peptides are common and likely to occur in the soil environment based on known protein structure and sequence data. GEOCHEM-PC (Parker et al. 1995) predicted that for a soil pH of 7.46, the net charge of Val=0, Glu=−2, Glu-Phe=−2, and Val-Pro-Pro=+2. Other selected substrate properties are given in Table 4. All radio-isotopes were purchased from ICN Pharmaceuticals (Irvine, CA, USA).

2.6 AA and peptide mineralization to CO_2

Soil samples (5 g) were weighed into 50 ml polypropylene tubes, and 0.5 ml of ^{14}C-labeled Val, Glu, Phe-Glu, or Val-Pro-Pro (9,990 MBq mmol⁻¹) solution was added dropwise to each soil type to reach a final concentration of 0.5 mM (2.7 kBq kg⁻¹). Any evolved $^{14}CO_2$ was trapped by placing a polypropylene vial containing 1 ml of 1 M NaOH above the soil, the sample tubes hermetically sealed with a rubber stopper and maintained at 25±0.5°C. The incubation temperature reflected soil temperature during the summer months, when plant growth is optimal. Respired $^{14}CO_2$ was quantified by removing the NaOH trap after 2, 5, 12, 24, 18, 96, or 168 h following addition of the ^{14}C label. After removal, the $^{14}CO_2$ amount trapped in NaOH was determined by liquid scintillation counting using a Wallac 1414 counter, and the aqueous compatible scintillation fluid Wallac Optiphase Hisafe 3 (EG&G, Milton Keynes, UK).

Table 3 Soluble C and N concentration of soil solutions (0–20 cm)[a] from three (OS, TS, CS) horticultural soils

Ratio	NO_3^--N (mg Nl^{-1})	NH_4^+-N (mg Nl^{-1})	DON (mg Nl^{-1})	DON % of TDN[b]	FAAs (mg Nl^{-1})	Protein (mg l^{-1})	DOC (mg l^{-1})	DOC:DON	Phenolics (mg l^{-1})	Humic substances (mg l^{-1})
OS	3.68±0.41b	0.64±0.12b	4.38±0.39a	50.4±4.9a	0.19±0.08b	3.01±0.42a	153.1±3.5b	12.3±2.1b	11.1±0.9c	46.1±3.7b
TS	4.73±0.39b	1.19±0.27a	1.68±0.27c	22.1±3.2b	0.22±0.02b	2.14±0.27b	107.4±4.2c	22.5±1.9a	16.8±2.1b	24.9±0.9c
CS	7.97±1.04a	1.27±0.20a	3.26±0.84b	26.1±2.7b	0.37±0.01a	1.59±0.19c	171.1±12.9a	13.7±2.1b	25.2±1.9a	58.5±4.3a

Values represent means±SEM ($n=3$). Means followed by different letters indicates significant differences at $P<0.05$ by Duncan's test procedures

DON Dissolved organic nitrogen, *TDN* total dissolved nitrogen, *FAA* free amino acid, *DOC* dissolved organic carbon

[a] Soil solutions were recovered from the soil by the centrifugal-drainage technique

[b] DON percentage over total dissolved N (TDN) in soil solution

At the end of the incubation period, the soil was shaken with 10 ml 0.5 M ice-cold K_2SO_4 for 10 min (250 rpm), and centrifuged for 5 min at 18,000×g. The supernatant was recovered for scintillation counting as described above. Substrate half-life was estimated using the double first-order exponential decay equation (Boddy et al. 2007), fitted by a least squares optimization routine to the mineralization data using Sigmaplot 8.0 (Systat Software UK, London, UK). Any ^{14}C label not recovered in either the NaOH traps or K_2SO_4 extracts was assumed to be in the microbial biomass (biomass-^{14}C). Microbial biomass carbon yields (Y) from the AA and peptide substrate were determined using the following equation: Y=biomass-^{14}C/(biomass-^{14}C+^{14}CO$_2$).

2.7 Inhibitor studies

Biochemical inhibitors and sterilization treatments were applied to soil samples to determine if AA and peptide breakdown is predominantly a biological process. The method is essentially identical to that described above, with the exception that glutamate, valine, Glu-Phe, or Val-Pro-Pro was added to the soil at a final concentration of 0.5 mM, and ^{14}CO$_2$ evolution was measured over a 24-h period. Incubations were carried out on untreated (control), autoclaved (1 h, 121°C), or CHCl$_3$-fumigated (24 h) soils. Alternatively, HgCl$_2$ (12 mM) and sodium azide (10 mM) were mixed

with ^{14}C-labeled AA and peptide solutions for 20 min prior to addition the untreated (control) soils.

2.8 AA and peptide sorption

Substrate sorption to the soil solid phase was determined using CHCl$_3$-fumigated soil (Joergensen and Mueller 1996) to prevent active microbial degradation of substrates during the sorption assays (Roberts et al. 2007). The protocol included placing 0.4 g CHCl$_3$-fumigated soil in 10 ml polypropylene tubes, and 2 ml of either a uniformly ^{14}C-labeled Glu (specific activity=9.7 GBq mmol^{-1}), Val (specific activity=2.1 GBq mmol^{-1}), Glu-Phe (specific activity= 2.1 GBq mmol^{-1}), or Val-Pro-Pro (specific activity= 2.1 GBq mmol^1) solution to the soil resulting in concentrations ranging from 0 to 2.5 mM, and total activity of 0.2 kBq. The soil and sorption solutions were orbitally shaken at 300 rpm for 10 min. After shaking, the soil supernatant was immediately centrifuged at 15,000×g, for 5 min, and the supernatant recovered for ^{14}C determination as described above. A Freundlich sorption isotherm (Roberts et al. 2007) was then fit to the experimental data using the following equation:

$$S = a \times \mathrm{ESC}^b \qquad (1)$$

where ESC is the equilibrium solution concentration at the end of the experiment (μM), S is the amount of solid phase

Table 4 Selected properties of AAs and peptides

Properties	Valine (Val)	Glutamate (Glu)	Glu-Phe	Val-Pro-Pro
Molecular formula	$C_5H_{11}NO_2$	$C_5H_9NO_4$	$C_{14}H_{18}N_2O_5$	$C_{15}H_{27}N_3O_5$
Molecular weight	117.15	147.13	294.31	329.34
Isoelectric point	6.00	3.08	4.28	7.64
Polarity	Neutral	Acidic	Alkaline	Basic
Dissociation constant (-COOH)	2.39	2.10	1.89	1.97
Dissociation constant (−NH$_2$)	9.74	9.47	9.84	10.24
charge	0	−2	−2	+2
C:N ratio	5:1	5:1	14:2	15:3

sorption (μM), and a and b are empirically derived parameters. Partition coefficients (K_d) were calculated as follows:

$$K_d = S / ESC \qquad (2)$$

2.9 Statistical analyses

All measurements were made in triplicate and reported as mean values with standard error. Statistical analyses were carried out using SPSS 10.5 (SPSS, Chicago, IL, USA). It was assumed the data represented two independent groups (management and substrate) for multiple comparisons tests. Normality within each group was tested using residuals (Anscombe and Tukey 1963) according to Shapiro and Wilk (1965). Levene's test (1960) on squared residuals was used to test homogeneity of variances. If one of the assumptions was violated, the data was transformed to conform to the assumptions before multiple comparison tests were performed (Fisher 1935).

3 Results

3.1 Soil characteristics

Farm management practice significantly ($P<0.05$) affected soil chemical characteristics (see Table 2). The highest total soil C levels (17.6 mg kg^{-1}), total N (1.5 mg kg^{-1}), exchangeable Ca (16.07 c mol kg^{-1}), Mg (5.53 c mol kg^{-1}), K (1.13 c mol kg^{-1}), Na (2.29 c mol kg^{-1}), and CEC (21.36 c mol kg^{-1}) were observed in the organically managed plots (OS), and followed the trend OS>CS>TS. Soil respiration was highest in OS and lowest in the CS treatment. Significant differences, however, were not observed in pH among the three soils ($P>0.05$).

3.2 Soil solution chemistry

Soil solution C and N concentrations in the three soils are shown in Table 3. The highest NO_3^--N, NH_4^+-N, FAAs, DOC, phenol, and humic substance values occurred in the conventionally managed plots (CS), however, the highest DON, and protein were detected in the organically managed plots (OS). Soil solution DON was significantly ($P<0.05$) different among soil types, comprised 26–50% TDN, and followed the order OS>TS>CS. DOC:DON ratios ranged from 12.3 to 22.5 and followed a TS>CS>OS series.

3.3 AA and peptide mineralization and half-life

The substrate-dependent mineralization of ^{14}C-labeled AAs (Glu, Val) and peptides (Glu-Phe, Val-Pro-Pro) to CO_2, and

their corresponding ^{14}C half-lives are shown in Fig. 1 and Table 5. Generally, the rates of substrate mineralization exhibited an initial rapid linear mineralization phase (0–12 h; linear regression r^2=0.72–0.99), followed by a secondary slower mineralization phase (see Fig. 1). The total Val and Val-Pro-Pro mineralized to CO_2 in OS (33–38% of the total) was significantly ($P<0.05$) lower than in the TS (37.5–43.1% of the total) and SC (41.7–49.3%) treatments; however, a significant difference in the total amount of Glu and Glu-Phe mineralization among OS, TS, and CS at the end of the experimental period was not observed ($P>0.05$). Typically, only small amounts of the ^{14}C label was recovered from the soil solution and exchange phase at the end of a 7-day incubation period (<3% of total ^{14}C-applied was recovered in a 0.5 M K_2SO_4 extract), suggesting only very small amounts of the substrate remained in the soil solution or on the exchangeable phase after 7 days.

Substrate half-life was largely independent of AA and peptide type addition (see Table 5). The average Val half-life was estimated at 10.6±2.3 h, while the Val-Pro-Pro, Glu, and Glu-Phe half-lives were 9.4±2.2, 7.1±1.9, and 6.7±1.5 h respectively. In addition, soil management practice significantly affected substrate half-life ($P<0.05$). The mean substrate half-life in the CS treatment was 2.3 times greater than the OS treatment and 1.5 times longer than in the TS treatment (see Table 5).

3.4 Microbial biomass C yield

The amount of AA C and peptide C immobilized in the microbial biomass following the addition of the substrates is shown in Fig. 2. In general, the majority of substrate C (ranging from 0.68 to 0.85) was immobilized in the microbial biomass, and only small amounts were mineralized to CO_2. The immobilization-to-mineralization ratio [microbial biomass C yield (MBCY)] was both substrate and soil management strategy dependent, and typically followed a Val-Pro-Pro (mean±SEM=0.82±0.01, CV=2.9%)≈Val (mean±SEM= 0.81±0.02, CV=3.2%)>Glu-Phe (mean±SEM=0.70±0.02, CV=4.1%)>Glu (mean±SEM=0.70±0.01, CV=2.1%) order. Overall, OS MBCY was significantly insensitive to substrate addition ($P<0.05$); however, TS and CS exhibited no significant difference in MBCY, with the exception of Val-Pro-Pro addition (see Fig. 2).

3.5 Microbial inhibitor effects

The evolution of $^{14}CO_2$ was significantly reduced by treatments designed to modulate soil microbial biomass size and activity (Fig. 3). $CHCl_3$ fumigation and autoclaving significantly reduced mineralization rates ($P<0.01$) and almost completely inhibited microbial activity with substrate

Fig. 1 Time-dependent removal of ^{14}C-labeled Val (**a**), Val-Pro-Pro (**b**), Glu (**c**), and Glu-Phe (**d**) from the soil solution in three horticultural soils (OS, TS, CS) and their subsequent utilization by the soil microbial biomass in the production of ^{14}C-CO_2. Standard errors were less than 5% of the mean value; therefore, only mean data points are shown ($n=3$). The legend is the same for all panels. In some cases, symbols are larger than error bars. *Different letters* indicate statistically significant differences between or among the three soils at the $P<0.05$ level; *NS* indicates the lack of significant differences, i.e., $P>0.05$. The initial AA and peptide concentration added to the soil was 0.5 mM
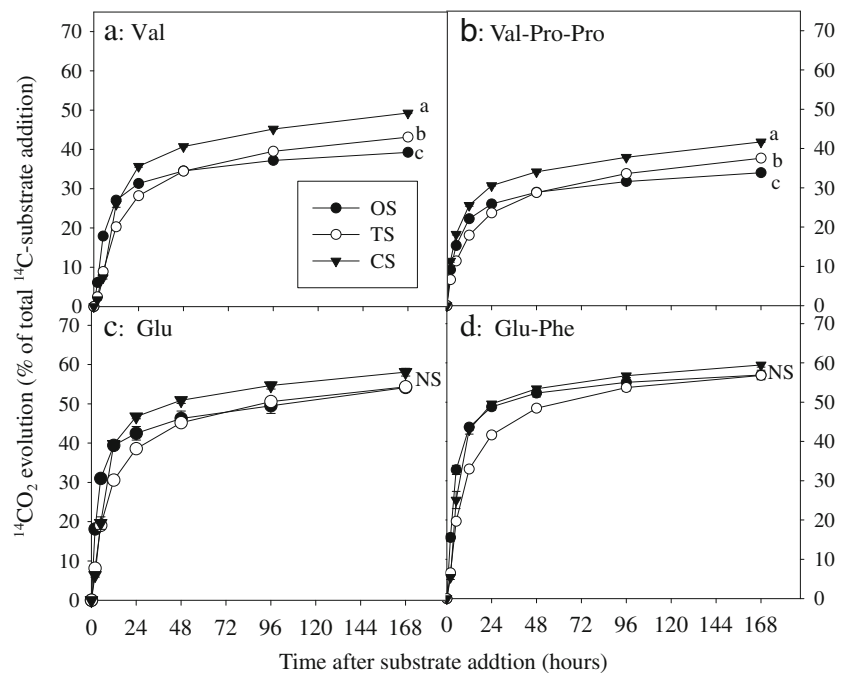
mineralization less than 2% of that observed in the control soils. The treatments involving the concurrent addition of Na-azide, $HgCl_2$ (chemical toxins), showed a significant and rapid reduction in mineralization rates, compared with the control ($P<0.05$). Generally, significant differences were not observed among the OS, TS, and CS treatments in response to the chemical toxins (see Fig. 3).

3.6 AA and peptide sorption

AA and peptide sorption to the soil's solid phase was concentration-dependent and followed a similar pattern in all three soils (Fig. 4). The amount of substrate sorption was significantly different among soil types and followed the series OS>TS>CS ($P<0.05$). AAs and peptides did not show a saturating sorption tendency over the concentration range (0–2.5 mM), which was an unexpected result. The Freundlich sorption isotherm equation showed good fit to the experimental results for both AAs and peptides ($R^2>0.951$). The mean soil values for the two Freundlich dimensionless *a* and *b* parameters were 0.39 and 1.49 for Glu, 0.10 and 2.23 for Val, 0.09 and 1.44 for Glu-Phe, and 0.72 and 1.29 for Val-Pro-Pro, respectively (Table 6). The solid phase

partition coefficients (K_d) exhibited some concentration dependency and consistently showed significantly increased concentration dependency for Val-Pro-Pro and decreased for Glu-Phe in all three soils (see Table 6).

4 Discussion

4.1 DON concentrations in different horticultural soils

The results of this study showed high DON concentrations in the three soil types, constituting 22.1% through to 50.4% of the TDN pool. Organic management soil (OS), which is known primarily for the application of organic fertilizer and the absence of artificial fertilizers, resulted in significantly higher DON amounts than that of conventional management soil (CS). This result is consistent with Ge et al. (2010a) for soluble organic N in soil solutions extracted from greenhouse and open-field horticultural soils. However, FFA amounts in soil solutions were very low, and only represented a small proportion of the total DON and DOC (<10% of total). To our knowledge, few studies have directly quantified the relative peptide amounts present in soil solutions,

Table 5 AA (Glu, Phe) and peptide (Glu-Phe, Val-Pro-Pro) half-lives in soil

Values represent means SEM ($n=3$). Means followed by different letters indicates significant differences at $P<0.05$ by Duncan's test procedures

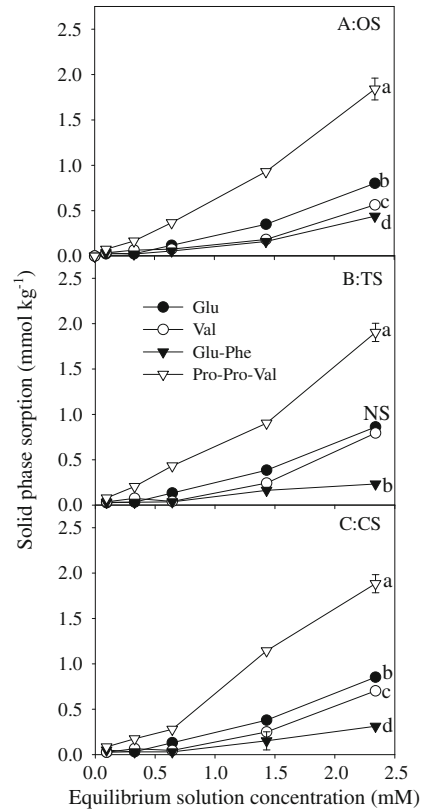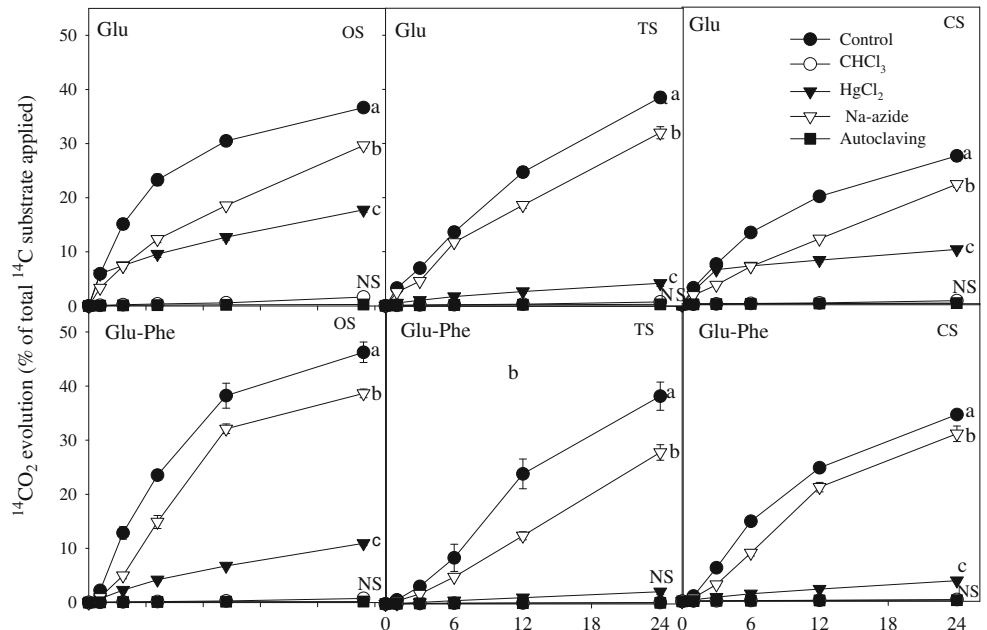| Soil	Val	Val-Pro-Pro	Glu	Glu-Phe	Means	CV (%)
OS	6.2±0.4c	6.7±0.1b	3.8±0.2c	4.2±0.3c	5.2±0.7a	27.5
TS	11.5±0.1b	7.5±0.4b	7.3±0.1b	6.2±0.1b	8.1±1.1b	28.6
CS	14.1±0.6a	13.9±0.7a	10.2±0.1a	9.6±0.6a	12.0±1.2c	19.9
Means	10.6±2.3b	9.4±2.2c	7.1±1.9b	6.7±1.5b	–	–
CV (%)	38.0	42.1	45.1	41.0	–	–
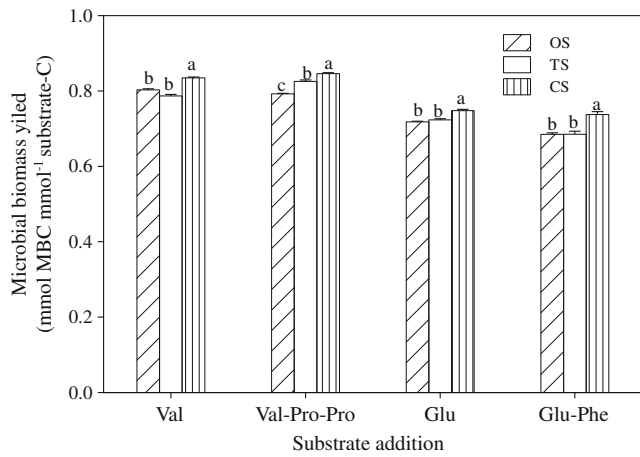
Fig. 2 Microbial biomass C yields (MBCY) as a function of time-dependent AA and peptide removal from the three horticultural soils (OS, TS, CS) following 168 h incubation. All values represent means±SEM ($n=3$)

although some studies have reported relative AA amounts present in soil polymeric and free states (Yu et al. 2002). Assessment of the soil N cycle concentration and turnover of upstream elements (e.g., proteins and polypeptides) can further support these results.

4.2 Soil AA and peptide characteristics

The rapid AA and peptide use reported in this work is consistent with previous studies that DON low molecular weight (LMW) components are not broken down by extracellular enzymes (e.g., deaminases) prior to uptake (Boddy et al. 2007). Our results clearly demonstrated that the mineralization and sorption of AAs and peptides is multifaceted,

Fig. 4 AA (Glu and Val) and peptide (Glu-Phe and Val-Pro-Pro) sorption on the solid phase of three horticultural soils: A OS, B TS, C CS. Data points are means±SEM ($n=3$). The legend is the same for all panels. In some cases, symbols are larger than error bars. Symbols represent experimental data points, and curves represent Freundlich isotherm fit to the experimental data (Roberts et al. 2007). *Different letters* indicate statistically significant differences between or among the three soils at the $P<0.05$ level; NS indicates the lack of significant differences, i.e., $P>0.05$

Fig. 3 Toxin (HgCl$_2$, 12 mM; sodium azide, 10 mM), soil sterilization (autoclaving, 1 h, 121°C), and CHCl$_3$ fumigation (24 h) effects on the time-dependent mineralization of ^{14}C-labeled AAs (Glu, 0.5 mM) and di-peptide (Glu-Phe, 0.5 mM) to ^{14}CO$_2$ in from three horticultural soils (OS, TS, CS). The control values represent unamended soil from sampled from the field. Values represent means±SEM ($n=3$). The legend is the same for all panels. In some cases, symbols are larger than error bars. *Different letters* indicate statistically significant differences between or among the three soils at the $P<0.05$ level; *NS* indicates the lack of significant differences, i.e., $P>0.05$

Table 6 Parameter estimates for the "Freundlich isotherm" equation of AA and peptide sorption to the soil's solid phase of three horticultural soils

Substrate	OS			TS			CS		
	a	b	R^2	a	b	R^2	a	b	R^2
Glu	0.23	1.64	0.998	0.72	1.27	0.997	0.23	1.56	0.997
Val	0.08	2.23	0.976	0.12	2.06	0.989	0.11	2.39	0.985
Glu-Phe	0.09	1.85	0.985	0.08	1.45	0.978	0.095	1.02	0.951
Val-Pro-Pro	0.68	1.36	0.997	0.76	1.23	0.985	0.72	1.27	0.988

a and b Empirically derived parameters, R^2 Residual sums of squares/total sum of squares in the dependent variate solution concentration (C_{sol}) Means followed by different letters indicates significant differences at $P<0.05$ by Duncan's test procedures (see Fig. 4 for curves)

with both the degree of sorption and mineralization-dependent on the individual AA and peptide species involved. The mean half-life in the three horticultural soils for AAs and peptides ranged from 6.7 to 10.2 h and followed a Val>Val-Pro-Pro>Glu-Phe>Glu order. Our mineralization results were very similar to the half-life values reported for other common soil LMW organic solutes, e.g., sugars, AAs, and organic acids (Boddy et al. 2007; Jones et al. 2005). This result also supports our argument that low free AA concentrations in soil solution are due to rapid microbial removal. A high turnover rate, combined with low intrinsic AA and peptide concentrations in soil solution suggests the AA and peptide rate of supply and not utilization is the rate-limiting step in the N cycle, at least in temperate agricultural soils (Jones et al. 2004). MBCY divergence between AAs and peptides at high substrate concentrations (0.5 mM) provided evidence that AA C and peptide C followed different metabolic pathways once inside the microbial cells. It also indicated that peptides are not extracellularly deaminated to AAs before subsequent uptake and use in the microbial cell. If the results were otherwise, the microbial C yield would have remained identical to that for the AAs, even if uptake were limited by external deaminase activity. Additional research is therefore required to determine microbial AA and peptide uptake kinetics, and their relationship to C and N supply and availability.

Previous studies have shown that DON LMW components may be fixed on clay surfaces (Amelung et al. 2002; Roberts et al. 2007). We did not determine the specific sorption sites in our soils; however, our experiments demonstrated that AAs and peptides can readily bind to the soil's solid phase, and that the saturation capacity may be large (>2.5 mM). Consistent with our results, Amelung et al. (2002) reported that most AAs and peptides are only weakly sorbed into the soil solid phase and are therefore relatively bioavailable. This is similar to a range of DON LMW components, which are also weakly held on the soil exchange phase, and can be readily desorbed using salts including 2 M KCl and 0.5 M K_2SO_4 (Jones and Willett 2006). Therefore, given the rapid mineralization of AAs

and peptides mentioned above, we can conclude that the soil microbial community primarily maintains the soil solution concentrations at low levels due to rapid uptake.

4.3 Influence of organic versus conventional management on AA and peptide mineralization in horticultural soils

Our results on LMW N turnover rates in different horticultural management soils suggested that agricultural management strategies have significant effects on the rate of soil AA and peptide mineralization. Organic management resulted in significant increases ($P<0.05$) in AA and peptide rates, possibly due to higher soil organic C (SOC) and soil respiration in OS than TS and CS. This may be due to increased biological and enzyme activity and microbial biomass resulting from organic matter application typical of organic management practices (Ge et al. 2011). Furthermore, under OS practices, where fertilizer is added as organic material (e.g., straw, animal excreta, and rapeseed cake), plants may obtain a larger proportion of N from organic sources, where the microbial N demand is rapidly saturated, rather than from N produced in native organic matter turnover. The activity of specific processes can be significantly influenced by the addition of organic residues, e.g., N mineralization (Campbell et al. 1999). Hopkins et al. (1997) reported the addition of farmyard manure (FYM) stimulated AA breakdown in soil. This is congruent with a study by Jones et al. (2005) that also showed short-term soil incubation with a complex organic residue (macerated wheat seeds) significantly increased AA mineralization rates.

Our results indicated that AA and peptide turnover rates in soils were correlated with a number of established soil quality parameters (e.g., SOC, soil respiration, NO_3^-). Therefore, we propose LMW N turnover and subsequent NH_4^+ production measurements in combination with determination of nitrification may provide an indication of potentially limiting steps in the soil N cycling rate (Kemmitt et al. 2008). It must be noted that our research was conducted in the laboratory and in the absence of plant roots, which affects microbial N cycling (Stienstra et al. 1994).

Consequently, similar research is required under field conditions to assess different soil and agricultural management regimes on the soil N cycling rate.

5 Conclusions

The results of our study indicated that soil management regimes have a substantial effect on AA and peptide behavior (mineralization, microbial uptake, and sorption, among other attributes) in horticultural production systems, although rapid AAs and peptides turnover appears to be an intrinsic feature of horticultural soils. Additional studies investigating the long-term functional significance of DON under different soil management regimes, particularly in organic practices, are therefore warranted.

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