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# Soil-based cycling and differential uptake of amino acids by three species of strawberry (*Fragaria* spp.) plants

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ABSTRACT

Evidence is growing that amino acids can be an important source of plant N in nutrient limited natural ecosystems, but relatively little is known about the effect of agricultural management on soil amino acid pools and turnover. Organic management in particular relies on slow-release organic inputs as fertilizer, which could result in greater pools of soil amino acids available for plant uptake. Moreover, we know little about potential differences in amino acid uptake ability within plant families and whether this ability may have been lost during domestication. In order to determine the relative effects of soil type and management on amino acid turnover, we measured the effect of fine- versus coarse-textured soil and organic versus conventional management on free amino acids and proteolytic activity in the field. Secondly, we conducted greenhouse experiments to determine the ability of domestic and wild strawberry to utilize amino acid-N. Fine-textured and organically managed soils contained significantly higher total C and N than coarse-textured and conventionally managed soils. There were no significant differences in free amino acids or protease activity in relation to texture or management. Amino acid turnover was calculated at 0.7–1.5 h. Turnover time was significantly greater in fine-textured soils. Turnover time as a result of substrate additions was significantly shorter in coarse-textured soils; in fine-texturedsoils turnover time was shorter under conventional management. This suggests less competition for amino acids in soils with greater C, N and/or cation exchange capacity (CEC), such as fine-textured and organically managed soils. Two wild species of strawberry, *Fragaria virginiana* and *Fragaria chiloensis*, took up significantly more $^{14}$C labeled glycine than the domesticated species, *Fragaria fragaria*. More research is needed to determine whether strawberry cultivars could be selected or bred for their ability to capture amino acid-N, thus improving N-use efficiency in farming systems relying on the breakdown of organic matter as a N source.

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## 1. Introduction

The question of whether plants can utilize organic nitrogen was asked as early as 1935 (Virtanen and Linkola, 1946). Methodology at the time was not sufficiently developed to determine whether all organic molecules were first mineralized before nutrient uptake. Recent research, however, has shown that many plants do have transporters for the uptake of organic forms of nitrogen, particularly amino acids (Soldal and Nissen, 1978; Jones and Darrah, 1994). In addition, plant uptake of organic nitrogen can be quite important to total plant production in nutrient limited systems such as the arctic tundra (Lipson and Näsholm, 2001). The significance of these findings to agriculture is controversial since agriculture uses great inputs of inorganic N and contains only small pools of free amino acids in the soil solution (Owen and Jones, 2001; Jones et al., 2005a).

Since organic and low-input agriculture rely primarily on green manures and/or composts and animal manures to meet crop N demands, amino acid-N may be present in greater supply in these systems. There is currently little published research on available pools and amino acid cycling in these systems. Relying on N mineralization from organic inputs can limit plant uptake and growth, especially at times of peak crop demand or in cool or dry soil conditions where mineralization is limited. Even small contributions of organic N to total N uptake at critical periods could improve yields in these systems. Moreover, crop varieties developed under high soluble N conditions could have inadvertently selected against the ability to use organic N. Murphy et al. (2007)

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showed that wheat cultivars selected under organic management perform better in organic systems, and cultivars selected under conventional management perform better in conventional systems. Selecting varieties with the ability to utilize a significant portion of their N as amino acid-N could be important when developing crops suited to organic and low-input systems.

The goal of this research was to compare amino acid pools and turnover in organic and conventionally managed strawberry fields and to determine amino acid uptake ability in two wild species and a domestic variety of strawberry. We tested the following hypotheses: (1) fine-textured and organically managed soils have higher concentrations of amino acids present in the soil solution than coarse-textured and conventionally managed soils; (2) amino acid turnover is slower in fine-textured and organically managed soils; and (3) wild species of strawberry take up more organic N than domestic varieties.

## 2. Materials and methods

### 2.1. Soil sampling and analyses

Soil was sampled in April 2005 at 0–10 cm from eight paired organic and conventional strawberry fields in the area of Watsonville, CA. Paired fields were carefully selected for the same soil type, strawberry variety, and all other environmental conditions except management, according to the method described by Reganold (1988). Surface soil texture of each field pair was either loamy sand or sandy loam (referred to as coarse-textured) or silty clay loam (referred to as fine-textured). Each sample consisted of 10–15 random subsamples, thoroughly homogenized. Soils were shipped on ice by overnight mail and analysis proceeded immediately on soils stored at 4 °C until completion 15 days later.

The following tests used methods by Gavlak et al. (2003): soil texture (method S14.10); nitrate-N (method S3.30); and ammonium-N (method S3.50). Additional soil parameters were analyzed as follows: total C and N were measured by combustion using a Leco CNS 2000. Protease enzyme activities were measured in 1 g dry weight soil using casein as protein substrate for potential protease activity and no added substrate for native protease activity (Ladd and Butler, 1972). Samples were measured on a Perkin Elmer Lambda 2 UV/VIS spectrometer at 700 nm with tyrosine standards. Amino acids were extracted from 1 g dry weight soil in a 1:5 w/v ratio with either water or a weak organic acid solution of 0.1 M malic acid and 0.1 M acetic acid. Samples were shaken for 30 min, centrifuged, filtered through 0.2 μm nitrocellulose paper and frozen until amino acid quantification with a Hitachi F-3010 fluorometer using the OPAME procedure with mercapto-propionate (Jones et al., 2002). Amino acid turnover was calculated by dividing the pool of free amino acids in the soil (as extracted in water) by the flux (native protease activity) of amino acids (Schlesinger, 1997; Berthrong and Finzi, 2006). Turnover as a result of substrate addition was determined by dividing the pool by the potential flux (potential protease activity). Potential nitrification was measured as described by Schmidt and Belser (1994) using 10 g moist weight soil. Nitrate-N after 10 days incubation was measured on a Latchett QuickCHem FIA+8000 series autoanalyzer, using the $NH_4Cl_2$ and salicylate methods. All laboratory measurements were carried out in triplicate per field and averaged for statistical analysis.

### 2.2. Amino acid uptake

Coarse-textured soils sampled from both organic and conventionally managed fields described above were pooled and utilized for greenhouse experiments. Bulk density and water holding capacity of the soil were measured by filling 50 mL plastic test tubes (11.5 × 3.0 tapering to 0.5 cm) with 78 g dry soil and adding water slowly until water dripped out (20.75 mL); total dry weight divided by 50 mL is bulk density and weight of water held divided by weight of dry soil is water holding capacity. Three strawberry species *Fragaria chiloensis*, *Fragaria virginiana*, and *Fragaria fragaria* (cultivar: Tribute) were used in this study. The two wild species, *Fragaria chiloensis* and *F. virginiana*, were obtained from Plants of the Wild (Tekoa, WA) and the domestic species, *F. fragaria*, was obtained from the WSU Organic Farm. Strawberry plants were maintained in the greenhouse until ample runners were produced. Runners were selected from each of the mother plants and rooted in tubes in a humidity chamber in a greenhouse under natural light. Plants were watered as needed with a 100 μg $L^{-1}$ solution of Peter's 20-20-20 NPK Fertilizer (3.94% N as $NH_4^+$, 6.05% N as $NO_3^-$, 10.01% N as urea) plus 2 μg $L^{-1}$ STEM trace elements + Fe (Scotts-Sierra Horticultural Products Co, Marysville, OH). Plants were used in two laboratory experiments at approximately 3 weeks as described below.

#### 2.2.1. Experiment 1

Using intact plants in growth tubes, the surface of the tubes was sealed with melted paraffin wax and 3 mL $^{14}C$ labeled glycine solution injected through the wax in the following dilutions: 33.3, 16.5, 10.0 and 3.0 mM, which equaled 5.0, 2.5, 1.5, and 0.5 mM in soil solution or 18.0, 9.0, 5.4, and 1.6 μg glycine-N $g^{-1}$ soil. Three milliliters of $H_2O$ was injected into control tubes. The holes were then plugged with more wax and tubes placed in an open fume hood under grow lights (25 μmol $m^{-2}$ $s^{-1}$) at room temperature, in a completely randomized design with five replicates. After 24 h, shoots were removed into individual test tubes and dried at 60 °C. Roots were removed from the soil, washed twice in 0.02 mM $CaCl_2$ solution using a brush to remove soil particles, and finally sprayed with fresh 0.02 mM $CaCl_2$. Roots were placed in individual test tubes and dried as above. Dried samples were weighed before combustion in a Biological Oxidizer OX700 and $^{14}C$ measured using a Perkin Elmer liquid scintillation analyzer Tri-Carb 2900 TR. Background $^{14}C$ levels present in the controls were subtracted from treatment values and uptake of glycine calculated as μg glycine $g^{-1}$ of root or shoot material.

#### 2.2.2. Experiment 2

Tubes containing growing plants were sealed as above and injected with 3 mL $^{14}C$ labeled 10 mM glycine, 5 mM $^{15}N$ labeled $(NH_4)_2(SO)_2$ or 10 mM $^{15}N$ labeled $KNO_3$ with seven replicates per treatment. Each treatment contained equimolar amounts of all three constituents with one constituent labeled in each treatment. Final soil concentrations of glycine, $(NH_4)_2(SO)_2$ and $KNO_3$ equaled 1.5, 0.75 and 1.5 mM, respectively, or 5.4 μg N $g^{-1}$ soil. Control plants received 3 mL $H_2O$ only. After 24 h, the experiment was terminated and samples processed as above. After drying, samples were placed inside tin foil capsules and total N and $^{15}N$ determined on a Thermo Finnigan Delta Plus Advantage mass spectrometer.

### 2.3. Statistical analysis

Differences and similarities in soil parameters between treatment and soil type were tested using a split-plot incomplete block experimental design with whole plot as soil type and subplot as management. Experiment 1 was analyzed using the test for heterogeneity of slopes (Little et al., 1993). Experiment 2 was analyzed as a completely randomized design (CRD) with a two-way treatment structure (treatment and species). Shoot and root data were analyzed separately. All statistics were analyzed using the SAS system for Windows version 9.1 ANOVA and LSmeans (SAS Institute, Cary, NC). Data were checked for model assumptions and transformed as necessary using the natural log. Data for amino acid uptake did not meet model assumptions so that a non-parametric ranked ANOVA was used for data analysis. When data were