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Zinc–amino acid complexes are more stable than free amino acids in saline and washed soils

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ABSTRACT

Biodegradability of metal–amino acids (AA) complexes has remained undocumented. Such knowledge is necessary to assess role of AA in metal mobility and bioavailability in the soil environments. In this study, biodegradation of free glycine (Gly), glutamine (Gln), arginine (Arg) and histidine (His) was compared with their Zn complexes in a natural saline soil before and after washing. The soil samples were collected from 0 to 15 cm depth of a saline soil before and 7 days after washing. The heavy washing of the saline soils using low salinity irrigation water (EC < 2 dS m^{-1}) is a common practice applied by the farmers in the region before plant seeding. Mineralization of AA, revealed by CO_2 release (35–66% in saline soil and 20–42% in washed soil) after 48 h incubation indicating rapid degradation of AA in soil. Complexation with Zn significantly reduced mineralization of AA. In saline soil treated with AA, the cumulative release of CO_2 after 48 h incubation varied from 12.4 to 47.5 mg C kg^{-1}, while it was 5.8–37.9 mg C kg^{-1} in the same soil treated with Zn–AA complexes (ZnAAC). The effect of complexation with Zn on CO_2 release was dependent on AA type. Lower C mineralization and thus, higher stability of ZnAAC compared with free AA supports a conclusion that complexation of metal greatly decreases degradability of AA. According to the results, the effectiveness of complexation with proteinaceous AA in metal mobilization in soil has to be given greater consideration than that reported previously.

1. Introduction

Plants actively modify the rhizosphere environment by root exudates (Oburger et al., 2009). Among several substances identified in root exudates, sugars, amino acids (AA) and organic acids have drawn considerable interest due to their role in processes at the root–soil interface such as metal chelating and acidification of rhizosphere (Jones et al., 2004; Fageria and Stone, 2006). The concentrations of AA in soil solution are often much greater than those of trace elements (Brynhildsen and Rosswall, 1995; Schwab et al., 2008). Therefore, AA may play a significant role in forming complexes with metals. Amino acids form complexes with metal cations mainly through carboxylate (–COO) and amine ($-NH_2$) groups, thereby affect the mobility and bioavailability of metals to plant (Aravind and Prasad, 2005; Jones et al., 2004). This mechanism is particularly important in environments with low concentration of micronutrients such as Zn, where plant demand can only be met by mobilization of micronutrients from sparingly soluble pools (Dakora and Phillips, 2002). Zinc (Zn) is an essential micronutrient, and its deficiency is common in agricultural soils worldwide including the calcareous saline soils of central Iran (Khoshgoftarmanesh et al., 2010). In spite of often high total Zn concentrations in such soils, only a small fraction is available to plant. Alkaline pH, low organic matter content, salinity and high contents of calcium carbonate are the main factors causing low Zn availability in calcareous soils (Alloway, 2008). On the other hand, root exudates significantly affect availability to plant of Zn in rhizosphere (Aravind and Prasad, 2005). Accordingly, a possible reason for differential tolerance to Zn deficiency of cereals grown in Zn-deficient calcareous soils is variations in organic ligands released from roots (Kalaycia et al., 1999; Rasouli-Sadaghiani et al., 2011).

The effectiveness of Zn–AA complexes (ZnAAC) in plant Zn nutrition depends on the residence time in soil (Jones et al., 1994). Less biodegradability of ZnAAC in soils than the free AA can indicate significant contribution of the metal–AA complexation process in

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plant Zn nutrition. The mean residence times of AA in soils have been reported to be from less than 4 h to several hours for a mixture of 15 AA added to a sandy loam soil (Jones and Shannon, 1999). The mean residence times of glycine and glutamine added to a loamy alpine meadow soil was found to be 1 day (Lipson et al., 1999). Jones et al. (1994) found glycine, glutamic acid and lycine had little effect on mobilization of metal micronutrients in rhizosphere and attributed this to rapid microbial degradation. However, little is known about the degradability of metal–AA complexes. Brynhildsen and Rosswall (1995) reported that some metal complexes of citrate were not degraded by citrate-utilizing *Klebsiellaoxytoca* in a chemically well-defined medium. In a similar study, Francis et al. (1992) reported complexes of citrate with Ca(II), Cu(II), Fe(II), Pb(II) or U(IV) were not degraded by a citrate-degrading *Pseudomonas fluorscens* strain. Although the biodegradability of some metal–organic complexes has been studied in pure cultures, we did not find data in the literature on the degradability of metal–AA complexes in soil environments. Such knowledge is necessary for determining the role of AA in metal mobility and bioavailability in soil.

The biodegradation of AA in soil is a function of different factors including soil properties and climate factor. One major argument against the potential relevance of AA in mobility and bioavailability of metal is short residence time of AA in soil due to rapid degradation by the soil microbial community. We hypothesized that complexation with metals may negatively affect the degradability of AA. It has been documented that plant root is well equipped to recapture metal complexes in competition with a microbial population (Jones et al., 1994). Therefore, higher stability of ZnAAC than free AA can indicate greater contribution of AA to mobility and bioavailability of Zn for plant. In this study we evaluated our hypothesis by comparing the biodegradation of AA with their Zn complexes in soil. According to the previous studies (Rietz and Haynes, 2003; Sardinha et al., 2003; Wichern et al., 2006) soil salinity has a profound influence on soil organic matter (SOM) decomposition. Salt affected soils occupy more than 30% of the arable lands in Iran and represent a major limiting factor in crop production (Qadir et al., 2008). On the other hand, soil leaching is a practice commonly applied by the farmers in the central regions of Iran before plant cultivation. Soil washing reduces microbial activities through loss of microbial biomass and soluble organic matter (Kalbitz et al., 2000). Therefore, the aim of our work was to determine the degradation of AA and their complexes with Zn in a saline soil and a washed soil.

2. Materials and methods

2.1. Synthesis and characterization of ZnAAC

Zinc–AA complexes were prepared using four the AA glycine (Gly), glutamine (Gln), argenine (Arg) and histidine (His), the most abundant AA in soil (Jones et al., 2004; Rothstein, 2009; Werdin-Pfisterer et al., 2009), as complexing agents.

A solution of Gly, Gln, Arg or His (2 mmol) in 5 ml distilled water was slowly added to a solution of Zn(II) acetate (0.22 g, 1 mmol) in 2 ml distilled water. The mixture was heated at reflux temperature for 2 h while being stirred vigorously. After evaporation of the solvent at room temperature, white microcrystals of ZnAAC were recovered. The products were washed with cold ethanol, followed by diethyl ether and air-dried.

A Perkin–Elmer 2400 CHN elemental analyzer was used to quantify carbon (C), nitrogen (N), hydrogen (H) and oxygen (O) in various operating modes (CHN and oxygen modes). Atomic absorption measurements of Zn were recorded with an atomic absorption spectrometry (PerkinElmer 3030; PerkinElmer, Wellesley, MA, USA). The FT-IR spectra were measured with a FT-IR JASCO 460 spectrophotometer over KBr pellet in 4000–400 cm^{-1} range.

2.2. Soil sampling and analysis

The soil samples were collected from 0 to 15 cm depth of a saline soil, before and 7 days after in situ washing, at Rudasht Research Station (32° 29′ N; 52° 11′ E), Isfahan, Iran. The soil is classified as a Typic Haplocambid (Soil Survey Staff, 1999). The heavy washing of the saline soil with low-salinity irrigation water (EC < 2 dS m^{-1}) is a common practice applied by the farmers in the region before plant seeding. Soil washing was performed by ponding 10 cm of water (about 1000 m^3 ha^{-1}). Four replicate soil cores were pooled to make a composite sample. Sub-samples were air-dried, passed through a 2-mm sieve and stored at room temperature for physicochemical analyses. Other sub-samples were sieved without drying (field moist), incubated at 25 °C for 7 days and stored in polyethylene bags at 4 °C for microbial biomass and respiration analysis.

Soil pH was determined in saturation extracts using a Metrohm pH meter (Model 691, Switzerland). The electrical conductivity (EC) was measured in the same soil saturation extracts using a Metrohm EC-meter (Model Ohm-644, Switzerland). Soil texture was determined by the hydrometer method (Gee and Bauder, 1986). Total N was determined using Kjeldahl digestion (Bremner and Mulvaney, 1982), and the organic C content was analyzed by wet digestion (Nelson and Sommers, 1982). Total AA concentration was measured using the method of Rosen (1957): the soil samples were extracted with 2 M KCl (1:10 soil to solution ratio) for 1 h by shaking at 200 rpm and the extract was filtered through Whatman 42 filter paper. Aliquots of 1 ml of extracts were mixed with 0.5 ml cyanide-acetate buffer and 0.5 ml 3% ninhydrin solution in Methyl Cellosolve and heated for 15 min in a 100 °C water bath. Concentrations of AA were determined by comparing the optical absorbance (570 nm) of the samples relative to a standard curve prepared with leucine. Main soil characteristics are presented in Table 1.

2.3. Experimental design

To study AA degradation, C mineralization was determined in an incubation experiment with a completely randomized factorial design, in which the four amino acids (Gly, Gln, Arg and His) were added either as free AA or complexed with Zn to non-washed or washed soil. To separate AA complexation effects from Zn effects without complexation, C mineralization was also measured in control samples receiving no AA, but either only Zn, glucose, or Zn

Table 1
Characteristics of the saline soil and the washed soil.

Property	Saline soil	Washed soil
EC_e (dS m^{-1})	11.9	3.7
pH (H_2O)	7.6	7.7
SAR	15.1	1.1
Sand (%)	12.5	13.1
Clay (%)	41.7	40.3
$CaCO_3$ (%)	35.5	36.4
DTPA-extractable Zn (mg kg^{-1})	0.29	0.25
Total N (g kg^{-1} soil)	1.2	1.1
Organic C (g kg^{-1} soil)	4.83	4.78
Total amino acid (mmol kg^{-1} soil)	$0.94^a \pm 0.06$	$0.71^a \pm 0.06$
Basal respiration (mg CO_2 kg^{-1} d^{-1})	$36.3^a \pm 0.28$	$26.7^b \pm 0.52$
Microbial biomass C (mg kg^{-1} soil)	$96.6^a \pm 0.92$	$71.7^b \pm 0.64$
Microbial biomass N (mg kg^{-1} soil)	$36.0^a \pm 0.87$	$24.6^b \pm 0.35$
Arginine ammonification (mg NH_4–N kg^{-1} h^{-1})	$0.58^a \pm 0.02$	$0.29^b \pm 0.03$

Values are means ± standard error ($n = 3$). Means having different letters in the same row are significantly different at the 5% level by LSD. EC_e, Electrical conductivity of soil saturation extract; SAR, Sodium adsorption ratio.

Table 2
Analytical data for zinc–amino acid complexes (ZnAAC).

ZnAAC	Formula weight	Yield %	% Found (calc.)[a]				LogK_{st}[b]
			C	H	N	Zn	
$[Zn(Gly)_2]$	213.53	87.11	22.54 (22.50)	3.82 (3.78)	13.06 (13.12)	30.57 (30.63)	9.8
$[Zn(Gln)_2]$	355.69	80.69	33.94 (33.76)	4.98 (5.10)	15.47 (15.76)	18.12 (18.39)	8.64
$[Zn(Arg)_2]\cdot 0.5H_2O$	420.83	83.88	34.31 (34.25)	6.36 (6.47)	26.49 (26.53)	15.32 (15.54)	9.0
$[Zn(His)_2]\cdot H_2O$	391.73	83.22	36.66 (36.79)	4.89 (4.63)	21.53 (21.60)	16.36 (16.70)	13

[a] Theoretical percentage of the elements.
[b] Log value of the complex stability constant from database of the program MINEQL+ 4.6.

and glucose. The amount of Zn added to the soil in the Zn treatments without AA was equal to the amount added with ZnAAC in the treatments with complexes. All the experiments were performed in 3 replications. The total number of samples was 72.

2.4. Soil microbiological analysis

To study C mineralization, triplicates of 50 g soil were mixed with 5 ml solution of 10 mM AA [Gly, Gln, Arg, and His] or 5 mM ZnAAC [$Zn(Gly)_2$, $Zn(Gln)_2$, $Zn(Arg)_2$ and $Zn(His)_2$] and incubated for 48 h at 25 ± 1 °C. The final AA concentrations were chosen based on the total concentration of AA in maize root cells (10–20 mM) and likely concentrations present in the rhizosphere due to bursting of root epidermal cells (Jones et al., 2005). Distilled water was added to each soil sample prior to incubation to achieve a saturation of 50% water holding capacity. A vial with 10 ml solution of 0.1 M NaOH was placed on a platform above the soil sample in each jar. The trapping alkali solutions were replaced after 1, 2, 3, 6, 9, 12, 18, 24 and 48 h of incubation. Carbon dioxide released during incubation was determined by titration of alkali traps to a phenolphthalein endpoint, with 0.05 M H_2SO_4 following precipitation of carbonates by $BaCl_2$ solution (Alef, 1995). Soil sub-samples with no added AA or ZnAAC were used to determine basal respiration. The net mineralization rates were calculated by subtracting basal respiration rates from those of the respective AA and ZnAAC treatments.

To estimate AA half-life, a first-order kinetics equation was fitted to the cumulative amount of trapped CO_2 using Curve Expert 1.3,

$$C_m = C_0\left(1 - e^{-kt}\right),$$

where, C_m is the organic C mineralized at time t, C_0 is the potentially mineralizable organic C pool and k is the first order rate constant. The AA half-life ($t_{1/2}$) was calculated as follows:

$$t_{1/2} = 0.693/k$$

Arginine ammonification was measured using the incubation method of Lin and Brookes (1999). Six portions of moist soil (50% water holding capacity) equivalent to 3 g oven dried soil were taken. Three portions were amended with 3 ml of 0.5% Arg solution and incubated for 6 h at 30 °C. The soils were then extracted with 2.5 M KCl for 30 min by shaking at 200 rpm and filtered through Whatman 42 filter paper. The three other portions were extracted similarly directly after addition of the 0.5% Arg solution. The amount of NH_4–N in the soil extracts was measured by steam distillation (Keeney and Nelson, 1982) and Arg ammonification rates (mg NH_4–N kg^{-1} h^{-1}) calculated as the difference between incubated and non-incubated portion. This parameter is an indicator of microbial activity in soil, since most heterotrophs possess endocellularammonifying capacity, and it was found to be closely correlated with soil microbial biomass and activity (Alef and Kleiner, 1986).

Microbial biomass C and N were estimated using the chloroform fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987). Six portions of moist soil equivalent to 25 g oven-dry soil were taken from each soil sample. Three portions were fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$. Following fumigant removal, the soil was extracted with 100 ml 0.5 M K_2SO_4. The other three non-fumigated portions were extracted similarly at the time fumigation commenced. Organic C in the extracts was measured using a Shimadzu TOC-CS22 TOC Analyzer. Microbial biomass C was calculated as follows: microbial biomass C = E_C/k_{E_C}, where E_C = (organic C extracted from fumigated soils) − (organic C extracted from non-fumigated soils) and $k_{E_C} = 0.45$ (Brookes et al., 1985; Joergensen and Mueller, 1996).

Total N in the extracts was measured using the Kjeldahl method (Bremner and Mulvaney, 1982; Brookes et al., 1985). Microbial biomass N was calculated as follows: microbial biomass N = E_N/k_{E_N}, where E_N = (total N extracted from fumigated soils) − (total N extracted from non-fumigated soils) and $k_{E_N} = 0.54$ (Brookes et al., 1985; Joergensen and Mueller, 1996). Microbiological analyses were carried out in triplicate.

2.5. Statistical analysis

Treatment effects were determined by means of analysis of variance using general linear models (GLM). Means were compared using least significant differences (LSD) at $P < 0.05$ (SAS Institute, 2000).

3. Results

3.1. Characteristics of ZnAAC

The analytical data for ZnAAC are reported in Table 2. The results of elemental analysis support the composition of ZnAAC with a 2:1 ligand: metal molar ratio in consistent with their theoretical formulation $[Zn(L–L')_2]$. The molar ratio of AA to metal affects the biodegradability of AA–metal complexes. Complexes with AA: Zn molar ratios of 2:1 are the most stable ZnAAC in aqueous solution (Wilkinson, 1987). Therefore, all complexes used in the present study were synthesized at a 2:1 M ratio. The FT-IR spectra of the free AA and their Zn complexes suggest that the studied AA act as bidentate ligands, binding to Zn via one oxygen and one nitrogen atom and thus forming a chelate ring. The vibrations and assignments given in Table 3 suggest that the AA ligands existed as zwitterions in the solid state. Generally, the ν(C=O), ν(C–O), ν(NH_2), and δ(NH_2) of the AA ligands are affected by coordination to metal ions (Nakamato, 2009). We did not detect any strong band around 3300 cm^{-1}, which is characteristic for O–H functional group, neither in the spectrum of the free AA, nor of their Zn(II) complexes, indicating deprotonation of the carboxilic O–H group in the zwitterions and complexes.

The FT-IR spectra of ZnAAC show an absorption pattern in the 4000–400 cm^{-1} region that is similar to AA. Predominant

Table 3
Selected IR bands (cm^{-1}) of amino acids (AA) and their complexes with zinc (ZnAAC) (KBr disk).

AA or ZnAAC	$\nu(NH_2)$	$\nu(C{=}O)$	$\nu(C{-}O)$	$\delta(NH_2)$	$\delta(C{=}O)$
Gly	3100,2000	1596	1409	1502	503
$[Zn(Gly)_2]$	3306,3268	1599	1407	–	723
Gln	3267,3210	1634	1411	1200	–
$[Zn(Gln)_2]$	3267,3210	1645	1409	1686	777
Arg	3258,3056	1562	1474	1721	1200,600
$[Zn(Arg)_2]\cdot 0.5H_2O$	3139,3010	1594	1403	–	655
His	3126	1633	1455	1200,600	1200,600
$[Zn(His)_2]\cdot H_2O$	3178	1695	1407	–	799

vibrations for the ZnAAC are associated with $\nu(CO)$, $\nu(C{-}O)$, $\nu(NH_2)$, $\delta(NH_2)$, and $\delta(CO)$. The observed vibrational bands for $-NH_2$ groups around 3100–3350 cm^{-1} are very sensitive to the effect of intermolecular interaction in the solid state and these bands sometimes appear very broad. Also, it is difficult to discuss the strength of the Zn(II)–NH_2 bond from the $\nu(NH_2)$. In comparison to free AA, the vibration of N–H bands appears to be shifted toward higher frequency in the ZnAAC proving the involvement of the amine group in the complex formation. The carboxylate ion of AA coordinates to Zn(II) as a mono-dentate mode. The C=O groups of ZnAAC have approximately the same frequency around 1594–1695 cm^{-1} and it is seen that the $\nu(CO)$ is metal sensitive (Nakamato, 2009). The chelate effect explains the high complex stability constants (Table 2). High stability of these complexes in aqueous solution and soil was also found using electronic spectroscopy and NMR (in D_2O) methods (data not shown).

3.2. Biodegradation of AA and ZnAAC

Mineralization of AA was significantly affected as a result of their complexation with Zn (Table 4). In saline soil treated with AA, the cumulative release of CO_2 after 48 h incubation varied from 12.4 to 47.5 mg C kg^{-1} soil, while in the same soil treated with ZnAAC, the cumulative release of CO_2 was 5.8–37.9 mg C kg^{-1} soil. The mineralization of C added as AA at the end of incubation period was 35.4–66.0% in the saline soil and 20.8–42.7% in the washed soil (Table 5). In both saline and washed soils, the CO_2–C produced by microorganisms in the presence of AA ranked as Arg > Gln > His > Gly (Fig. 1). The degradation patterns of AA/ZnAAC in both soils were fitted by the first-order kinetics equation. The half life ($t_{1/2}$) of the AA ranged between 7.4 and 12.1 h in the saline soil and between 9.5 and 13.5 h in the washed soil (Table 5). The $t_{1/2}$ of the ZnAAC were 8.0–24.2 and 11.9–26.2 h in the saline and washed soils, respectively.

Significant differences were found between the saline soil and washed soil in the CO_2 release (Table 4). In the saline soil, the cumulative C mineralized as CO_2, expressed as a percentage of organic C in AA, within 48 h incubation ranged from 35.4% for His to 66.0% for Arg-amended soil, whereas in the washed soil varied from 20.8% for His to 42.7% for Gly treatment (Table 5). After 48 h, 17.6%–52.7% of the total organic carbon added to the saline soil in the form of $[Zn(His)_2]$ and $[Zn(Arg)_2]$ were mineralized, respectively. In the washed soil, the amount of organic C evolved ranged from 14.5% for $[Zn(His)_2]$ to 24.6% for $[Zn(Gln)_2]$.

The microbial biomass C and N in the washed soil decreased by 35.0 and 46.3% compared to the saline soil, respectively (Table 1). The Arg ammonification rate in the saline soil (0.58 mg NH_4–N kg^{-1} h^{-1}) was also higher than that in the washed soil (0.29 mg NH_4–N kg^{-1} h^{-1}).

In both saline and washed soils, the increase in cumulative CO_2 production by addition of glucose was similar in with and without added Zn treatments. In the saline soil, the mineralization of glucose in with and without added Zn treatments were 102.9 and 99.9 mg C kg^{-1} soil, respectively (Fig. 2). In the washed soil, the mineralization rate of C for the glucose was 86.4 mg C kg^{-1} soil in with added Zn treatment and 84.4 mg C kg^{-1} soil in without added Zn treatment. In both soils, the cumulative release of CO_2 after 48 h incubation was similar between with and without added Zn treatments (Fig. 3).

Table 4
Analysis of variance of organic C mineralization (C_m), percentage of added organic C evolved as CO_2–C (C_p) and half life of four amino acids as free or complexed with zinc in saline and washed soil during 48 h incubation.

Source of variation	df	Mean square		
		C_m	C_p	Half life
Soil (S)	1	2011.6^{***}	4309.2^{***}	60.5^{***}
Amino acid (AA)	3	1188.2^{***}	1019.7^{***}	222.1^{***}
S × AA	3	354.9^{***}	574.3^{***}	8.8^{***}
Complexation (C)	1	503.9^{***}	2082.9^{***}	305.1^{***}
C × S	1	28.1^{**}	79.6^{***}	0.82
C × AA	3	18.7^{**}	185.7^{***}	86.1^{***}
C × S × AA	3	10.5^{*}	20.6^{***}	1.1

$^{*}P < 0.05$.
$^{**}P < 0.01$.
$^{***}P < 0.001$.

Table 5
Percentage of added organic C evolved as CO_2–C (C_p) and half life of amino acids (AA) after 48 h incubation.

AA or ZnAAC	Saline soil		Washed soil	
	C_p (%)	Half life (h)	C_p (%)	Half life (h)
Gly	$51.6^{c} \pm 1.46$	$8.4^{d} \pm 0.11$	$42.7^{a} \pm 1.66$	$9.5^{e} \pm 0.23$
$[Zn(Gly)_2]$	$24.4^{e} \pm 1.54$	$15.8^{b} \pm 0.21$	$21.8^{d} \pm 1.07$	$15.4^{b} \pm 0.22$
Gln	$56.8^{b} \pm 1.87$	$7.4^{e} \pm 0.16$	$30.4^{b} \pm 1.93$	$10.1^{ed} \pm 0.24$
$[Zn(Gln)_2]$	$52.1^{c} \pm 1.64$	$8.0^{ed} \pm 0.12$	$24.6^{c} \pm 1.42$	$11.9^{cd} \pm 0.29$
Arg	$66.0^{a} \pm 0.40$	$7.4^{e} \pm 0.10$	$29.8^{b} \pm 1.30$	$11.8^{cd} \pm 0.18$
$[Zn(Arg)_2]\cdot 0.5H_2O$	$52.7^{c} \pm 0.67$	$8.5^{d} \pm 0.15$	$20.4^{e} \pm 0.99$	$12.1^{c} \pm 0.30$
His	$35.4^{d} \pm 0.13$	$12.1^{c} \pm 0.18$	$20.8^{ed} \pm 0.49$	$13.5^{bc} \pm 0.23$
$[Zn(His)_2]\cdot H_2O$	$17.6^{f} \pm 0.61$	$24.2^{a} \pm 0.36$	$14.5^{f} \pm 0.41$	$25.2^{a} \pm 0.32$

The C_p was calculated by subtracting the total organic C mineralized in the non-amended soil from that mineralized in the AA- or Zn–AA complexes (ZnAAC)-amended soil and dividing the result by the amount of organic C in the AA or ZnAAC added to the soil. All values are means ± standard error ($n = 3$). Means having different letters in the same column are significantly different at the 5% level by LSD.

4. Discussion

4.1. Biodegradation of AA

The net mineralization rates calculated by subtracting the control respiration values from those of the AA induced respiration varied upon AA type (Fig. 1a–d). The wide difference in CO_2 efflux from the various AA treatments were related to different amounts of C added to soils (1.2 mg C for Gly, 3 mg C for Gln, and 3.6 mg C for Arg and His) by adding the same AA concentrations (10 mM). The addition of AA with a higher C content resulted in greater CO_2 production over the 48 h incubation period, with the exception for His. Although Arg and His added similar amount of C (3.6 mg AA–C) to soils, they resulted in different rates of CO_2 production, suggesting differences in either their microbial transport or metabolism. Results obtained from pure bacterial culture experiments showed that AA are transported into the cell by specific transporters (e.g., neutral, basic or acidic) (Anraku, 1980). The metabolism of AA is also different from each other (Vinolas et al., 2001). It has been shown that the mineralization of some AA is regulated by their relative position along the biosynthetic pathways, with AA present at the end of a biosynthetic pathway (e.g., lysine) are less

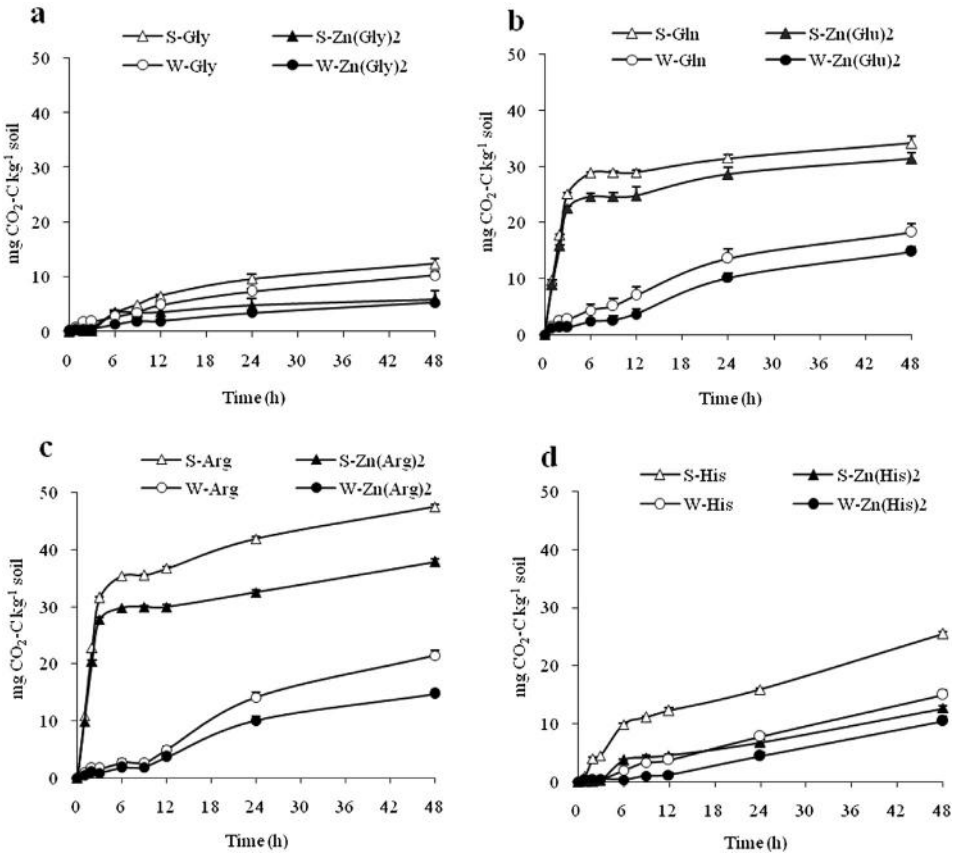

Fig. 1. Net decomposition of free a) glycine (Gly), b) glutamine (Gln), c) arginine (Arg) and d) histidine (His) and their complexes with Zn in saline (S) and washed (W) soils over time. The net mineralization rates were calculated by subtracting the control respiration values from those of the amino acids (AA) and Zn–amino acid complexes (ZnAAC) induced respiration. Data are means ± standard error ($n = 3$).

amenable to being metabolized in comparison to those present at the center (e.g., glutamate) (Roberts et al., 2009; Vinolas et al., 2001). High rate of glutamic acid decomposition compared to glucose or organic acids in the rhizospshere was reported by Renella et al. (2007). According to the results obtained from the previous studies, the intrinsic chemical and physical properties of AA (e.g., C:N ratio, molecular weight and aromaticity), have little influence on the microbial uptake of AA or subsequent rate of N mineralization. For example, McLain and Martens (2005) reported there were no significant differences in CO_2 production between the hydrophilic and the hydrophobic AA. Therefore, the rapid mineralization of Arg in our experiment (Fig. 1c) is possibly due to its low internal demand and its location at the end of a biosynthetic pathway. Arginine can be degraded by several microbial species through different metabolic pathways (Alef and Kleiner, 1986; Lin and Brookes, 1999). The changes in the rhizosphere microbial

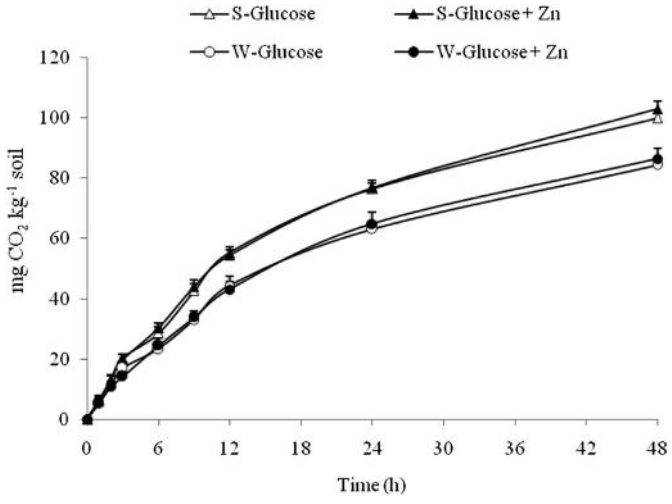

Fig. 2. Mineralization of glucose in the presence and absence of added Zn in saline (S) and washed (W) soils over time. Data are means ± standard error ($n = 3$).

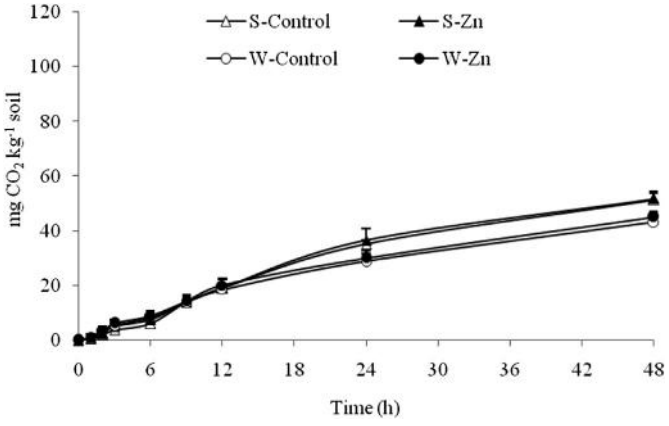

Fig. 3. Mineralization of C in the presence and absence of added Zn in saline (S) and washed (W) soils over time. Data are means ± standard error ($n = 3$).

communities upon release of N-rich root exudates were reported by Landi et al. (2006). Rapid ammonification of Arg has also been reported by Roberts et al. (2009).

It should be noted that we did not use radio- or stable-isotope-labeled AA to directly distinguish CO_2 produced from added AA vs. SOM, instead calculating these by difference from a control treatment. The CO_2 flux data and calculated half lives in the present study were in agreement with what reported by isotopically labeled AA addition experiments (Jones et al., 2005; Jones and Shannon, 1999). If CO_2 derived from native soil organic matter was significant, the half-lives were much lower than expected. Theoretically, priming is thought to result when high C:N ratio labile compounds are added to soil. Addition of high C:N ratio compounds stimulates soil microorganisms to breakdown low C:N ratio soil organic matter (Fontaine et al., 2003). In the present study, we added N rich substrates to the soil. Therefore, it would not be expected to observe a priming effect.

4.2. The effect of Zn complexation on the biodegradation of AA

Carbon mineralization was lower for ZnAAC than that for AA in both soils (Fig. 1), indicating that ZnAAC were less decomposable than free AA (Table 4). Lower biodegradability of metal–organic complexes in comparison with the corresponding free chelating agents has been reported in pure culture experiments (Brynhildsen and Rosswall, 1989; Joshi-Tope and Francis, 1995; White and Knowles, 2000. One factor which may limit the biodegradation of ZnAAC is uptake into the bacterial cell. The uptake of AA is affected by the presence of divalent cations. However, it has been reported bacteria discriminate between ligands and metal–ligand complexes in uptake processes. Francis et al. (1992) reported a lower ability of microorganisms to degrade heavy metal–organic complexes in comparison with free ligands and that this could be due to a lower affinity of enzymes for metal-complexed than for free organic ligands. Puzon et al. (2008) showed that *Ralstonia eutropha* JMP 134 and *Pseudomonas aeruginosa* pAO1 degraded malate, citrate, and His much faster than in absence than in presence of Cr(III) because malate dehydrogenase and AA oxidase could not use malate–Cr(III) and His–Cr(III), respectively.

Biodegradability of ZnAAC varied upon the type of AA (Table 5). In both soils, the $t_{1/2}$ of $Zn(Gly)_2$ and $Zn(His)_2$ was about 2 times higher than free Gly and His. In contrast, complexation with Zn had no significant effect on the $t_{1/2}$ of Gln and Arg probably due to lower stability of $[Zn(Gln)_2]$ and $[Zn(Arg)_2]$ complexes. The limiting step to biodegradation of metal–AA complexes has generally been identified as complex dissociation. Free ligands are much easier taken up by bacteria than metal–organic complexes (Vandevivere et al., 2001), explaining why complexes with a high stability constant tend to be much less amenable to biodegradation than complexes with a relatively low stability constant. Studies with nitrilotriacetic acid (White and Knowles, 2000), EDTA (Thomas et al., 1998), citrate (Brynhildsen and Rosswall, 1995), and acetate (Renella et al., 2004) showed that their biodegradation was strongly dependent on the type of metal present. Witschel and Egli (1998) reported [S,S]-EDDS was biodegraded in presence of metals only if they did not form very stable complexes with this compound. Also in our study, a negative correlation was found between complex stability constant and biodegradability, as $[Zn(His)_2]$ complex with the highest stability constant (Table 2) was lesser degraded (Table 5). In addition, $[Zn(Gln)_2]$ and $[Zn(Arg)_2]$ complexes have similar stability constants (8.64 and 9.0, respectively) and their biodegradability in saline soil was similar.

The fact that similar amounts of glucose was decomposed in combination with and without Zn, suggests that the differences in C mineralization between AA and ZnAAC are a result of AA complexation by Zn and not due to a toxicity effect of Zn. Lower C mineralization and thus, higher stability of ZnAAC compared with free AA supports a conclusion that complexation of metal greatly decreases degradability of AA. The reduction of AA decomposition as a result of complexation with Zn indicates that in previous studies (e.g., Jones et al., 1994) the contribution of AA in mobility and bioavailability of Zn to plant has been largely underestimated. Considering that plant roots can absorb AA–metal complexes in competition with microbial population (Jones et al., 1994), the effectiveness of complexation with proteinaceous AA in metal mobilization from soil environment and further uptake by plant roots has to be considered much greater than that reported previously.

4.3. The effect of soil washing on biodegradation of AA and ZnAAC

Our results suggest that soil washing significantly affected AA and ZnAAC turnover, and reduced their biodegradation (Fig. 1a–d). The remarkably low Arg ammonification rate in the washed soil indicates an inhibitory effect of soil washing on microbial activities. This is probably due to the loss of microbial biomass and soluble organic matter during soil washing, as confirmed by the lower microbial biomass C and N values in the washed soil (Table 1). These results are in contrast to other studies that have reported reduced microbial activities and organic carbon decomposition in salinized soil (Sardinha et al., 2003; Wichern et al., 2006; Yuan et al., 2007). This controversy is partly due to different experimental conditions, particularly with respect to the method of establishing different soil salinity levels. Natural and artificial salinity may have different effects on the decomposition of SOM. When considering negative effects of soil salinization on soil microbial biomass and functions (Egamberdieva et al., 2010), it should be considered that also the common practice of soil washing affects soil microbial biomass and activity.

Soil respiration was higher in the non-washed soil probably because of the larger microbial biomass (Table 1). In addition, lower amount of total AA concentration in washed soil indicated possible N loss from soil. In this study, the responses were studied in soils collected 7 d after washing, and without longer studies no information of the persistence of the washing impact or on the recovery of the soil microbial communities can be drawn. Further research is needed to investigate the effect of soil washing on the biodegradability of AA and ZnAAC follow up sampling over time.

5. Conclusion

Soil washing had a significant negative effect on soil microbial biomass and activity. Our observation of lower C mineralization with ZnAAC compared with AA supports a conclusion that Zn complexation decreased the degradability of AA, allowing more ZnAAC to stay in soil. The reduction of AA decomposition as a result of complexation with Zn implies that metal complexation may play an important role in mobility and bioavailability of Zn in the soil environment. However, these views need to be tested in the future studies. It may be important to monitor the changes in microbial diversity and identify the microbial groups that are responsible for the biodegradation of these molecules. The use of ^{14}C-labeled metal complexes may also allow better discrimination of the fate of the metal complexes that are not mineralized, but may be incorporated into the SOM.

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