SOIL SCIENCE ANNUAL
2023, 74(1), 161147

https://doi.org/10.37501/soilsa/161147

L-arginine application triggered soil hydrolytic activity

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Received: 2022-08-23
Accepted: 2023-02-11
Published online: 2023-02-12
Associated editor: J. Lasota

Keywords:

Protease
Hydrolases
Amino acid
Biodegradation
Forest soil
Fluorescein diacetate

Abstract

Application of amino acids to soils is reportedly associated with controversial responses in soil enzyme activities. The effects of L-arginine application on the fluorescein diacetate (FDA) hydrolysis and protease activity in an oak forest soil was investigated. The FDA hydrolysis and protease activity were regularly measured over a standard incubation period. The addition of L-arginine increased both FDA hydrolysis and protease activity after a lag time of 10 days. After 30 days, the ratio of FDA hydrolysis and protease activity in L-arginine-amended soil samples to those in the control reached 2.0 and 3.7, respectively. Moreover, FDA hydrolysis was found significantly ($r = 0.67$, $P < 0.05$) correlated with protease activity. It was concluded that L-arginine was able to stimulate FDA hydrolysis and protease activity, thereby making the soil hydrolytic system capable of facing more complicated substrates.

1. Introduction

An interminable variety of hydrolases is involved in energy transfer as well as C and N cycling in soil (Criquet et al., 2002; Andersson et al., 2004). However, the vast variety of these enzymes are difficult to determined directly (Schloter et al., 2003). A critical understanding of soil biological processes may, therefore, be acquired by measuring total microbial activity (Schnürer and Rosswall, 1982; Adam and Duncan, 2001).

The fluorescein diacetate (FDA) hydrolysis is a common method that has been used to estimate total microbial activity in soil (Schnürer and Rosswall, 1982; Adam and Duncan, 2001; Green et al., 2006). Fluorescein diacetate (FDA) is non-specifically hydrolyzed by a wide range of hydrolytic enzymes such as protease, lipase, and esterase (Adam and Duncan, 2001; Green et al., 2006). The soil bacterial and fungal biomass was demonstrated to be significantly associated with the FDA hydrolysis assay. (Schnürer and Rosswall, 1982; Adam and Duncan, 2001; Gaspar et al., 2001). Moreover, FDA hydrolysis has a positive relationship with soil organic carbon and nitrogen content (Gaspar et al., 2001) and is sensitive to organic matter amendment quality (Sánchez-Monedero et al., 2008) and land management (Perucci, 1992; Haynes, 1999). The FDA hydrolysis rate responds positively to both organic (Perucci, 1992; Sánchez-Monedero et al., 2008) and mineral nitrogen additions to soil (Graham and Haynes, 2005). Thus, the FDA hydrolysis rate might have a relationship with additional simple organic N sources like amino acids. However, there is much less information about FDA hydrolysis responses to available C and N sources with low molecular weight (e.g., NH_4^+, amino acids) in soil.

Amino acids are generally considered a small portion of the dissolved organic nitrogen content in soil with a very fast turn-over, thereby playing a major role in N acquisition from soil (Jones et al., 2005). Microbial communities rapidly consume amino acids (Jones et al., 2008) due to either energy or C or N limitation in soil (McMahon and Schimel, 2017). Soil microbes preferentially used amino acids as C sources in a catabolic way (respiration) for increasing their biomass proliferation (Jones et al., 2008; Halsey et al., 2017), so that new generations of microbes would face nutrient deficits after consuming easy-to-degrade N and C sources. To compensate for low C concentrations, they presumably produce extracellular enzymes (e.g., protease) to use more complex organic substrates, such as proteins (Geisseler and Horwath, 2008). Many hydrolytic enzymes like protease (Allison and Vitousek, 2005), phosphatase (Renella et al., 2007) have positively responded to the addition of simple carbon and nitrogen sources to the soil. Fluoresceine diacetate (FDA) can be hydrolyzed by both intracellular and extracellular microbial enzymes and represents metabolically active soil microbial biomass (Lundgren, 1981). Moreover, FDA hydrolysis had a positive relationship with glucose-induced respiration in the litters (Stubberfield and Shaw, 1990). Therefore, the FDA

hydrolysis rate may have a relationship with new microbial enzymatic products that increase in response to additional C and N sources. It was hypothesized that addition of L-arginine to soil could stimulate FDA hydrolysis to enhance overall soil hydrolytic capabilities and, thereby, realize its potential for protein hydrolysis. This motivated the present study to investigate both general soil hydrolytic activity as indexed by FDA hydrolysis and protease activity.

2. Materials and Methods

For the purposes of this study, soil samples were collected from a depth of 0–15 cm at Delvara forest, west-central Iran (31°18´N, 51°18´E). The mean annual temperature and precipitation were 15.6°C and 303 mm, respectively. The forest area is naturally covered by oak (*Quercus brantii* Lindl). The presence of jungle is crucial in arid and semi-arid ecological region and finding a jungle with a considerable amount of plant debris and high-quality soil organic carbon is beneficial for having a diverse and dynamic soil microbial community structure. For soil sampling, three points with equal distance from each other (50 m) were selected. At each point, 10 soil cores within 2 m radius were collected and mixed as a composite sample. The samples were collected at June that nearly air-dried. Thus, the soil sample air-dried for 24 h at 25°C, passed through a 2-mm sieve and kept in polyethylene containers at room temperature before analysis. The soil was generally characterized as containing 405 g kg^{-1} sand, 280 g kg^{-1} clay, 13.8 g kg^{-1} organic C, 1.7 g kg^{-1} total N, and 520 g kg^{-1} calcium carbonate equivalent with a pH of 7.4. Soil pH were determined in the 1:2 soil: water ratio. Soil organic carbon was measured by wet digestion. Soil texture was determined by the pipette method. The calcium carbonate equivalent was measured by the titration method (Burt, 2004). Total N was determined with the Kjeldahl digestion procedure (Bremner, 1996).

For analysis, the samples were moistened with distilled water to 55% of their water holding capacity before pre-incubation at 25°C for seven days to stabilize biological activities (Renella et al., 2007). The pre-incubated soil samples were then treated with L-arginine at 0 and 50 μmol g^{-1} soil and incubated for another 40 days. During the incubation period, FDA hydrolysis and protease activity were measured periodically at 0, 1, 2, 3, 5, 10, 15, 20, 25, 30, 35, and 40 days. The FDA hydrolysis was measured by the technique described in Green et al. (2006). Briefly, 0.5 g of soil (dry weight) with 50 ml of Tris buffer (0.1 M, pH 7.6) and 0.5 ml of fluorescein diacetate solution (4.9 mM) were mixed then incubated for 2 h at 37°C. After incubation, 2 ml of acetone was added to the mixture and the soil suspension was then filtered using a Whatman No. 2 filter paper. The fluorescein color intensity was determined by using a spectrophotometer (PD-303; APEL CO., LTD. Japan) at 490 nm (Green et al., 2006). The protease activity was determined by method described in Alef and Nannipieri (1995). Briefly, a mixture of 1 g of soil with 5 ml of Tris buffer (50 mM, pH 8.1) and 5 ml of sodium caseinate (2%) incubated in a shaker water bath at 50°C for 2 h. 5 ml of 15% Trichloroacetic acid (TCA) was used to precipitate remaining casein. The 0.5 ml of clear supernatant (centrifuged at 13000 rpm for 2 min) was mixed with 0.75 ml of an alkaline reagent and 0.5 ml of Folin-Ciocalteu reagent (33%) in cuvette. After 1 hour, the Tyrosine concentration was determined by using a spectrophotometer (PD-303; APEL CO., LTD. Japan) at 700 nm Alef and Nannipieri (1995).

The statistical analysis was performed by SAS (Version 9.4), using general linear model (GLM) and Corr procedure for analysis of variance and correlation analysis respectively. The amino acid concentration and incubation time effects on fluorescein diacetate (FDA) hydrolysis rate and protease activity were analyzed by using two-way ANOVA in completely random design. The mean comparison was performed using the Least Significant Difference test (LSD, $P < 0.01$).

3. Results and Discussion

Fluorescein diacetate (FDA) hydrolysis decreased during the first five days of incubation. However, L-arginine amendment had no effect on it because significant differences were not observed between control and 50 μmol g^{-1} L-arginine treated samples. Therefore, it is assumed that FDA hydrolysis failed to respond to L-arginine application during the first 10 days of incubation. Beyond this time, however, significantly high FDA hydrolysis rates were observed in L-arginine-treated samples, which remained high up to the end of the incubation period ($P < 0.01$). At day 30, the FDA hydrolysis reached the maximum level and its ratio in the L-arginine-amended soil samples to that in the non-amended ones gradually reached 2.0 to decline thereafter up to the end of the period (Fig. 1a).

Protease activity remained unaffected during the first 10 days of L-arginine application. Since day 10, protease activity gradually increased, picked at day 30 and remained at greatest activity for 5 days, and decreased to the end of incubation (Fig. 1b). From day 10 up to the end of the incubation period, however, significant ($P < 0.01$) differences were observed in protease activity between the L-arginine-amended samples and the control ones (Fig. 1b). The ratio of protease activity in the L-arginine-amended samples to that in the control reached 3.7 at day 30. Interestingly, not only did both FDA hydrolysis and protease activity experience a 10-day lag time but both indices picked up at day 30 of the incubation period. This was also reflected in the significant correlation ($r = 0.67$, $P < 0.5$) established between FDA hydrolysis and protease activity (Fig. 2).

The observed increase in FDA hydrolysis in response to L-arginine corroborates the results reported elsewhere indicating enhanced FDA hydrolysis due to inorganic N application (Graham and Haynes, 2005), increased soil N content (Gaspar et al., 2001), and increased soil organic carbon (Haynes, 1999). Increases in soil organic C and N contents have been suggested to stimulate microbial growth and activity, thereby, leading to enhancements in FDA hydrolysis (Perucci, 1992; Haynes, 1999). Sánchez-Monedero et al. (2008) demonstrated that either stabilized or raw waste material amendments increased FDA hydrolysis during 60 days of incubation, lending further support to the gradual increase in FDA rate observed in the present study after

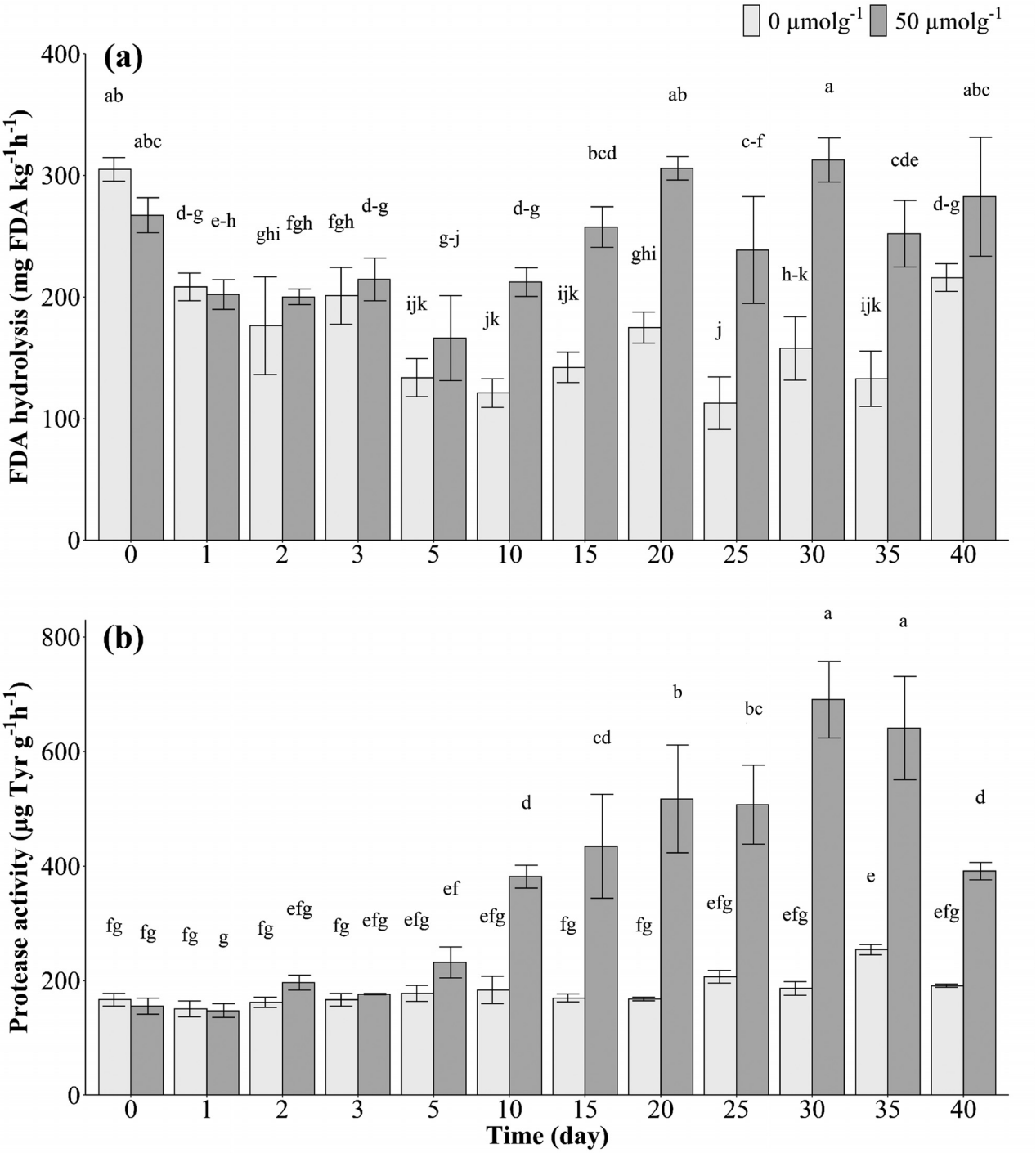

Fig. 1. The fluorescein diacetate (FDA) hydrolysis rate (a), and protease activity (b) in Delvara forest soil treated by 0 and 50 µmol g^{-1} concentrations of L-arginine during 40 days of incubation. Different letters represent significant difference (LSD, $P < 0.01$).

10 days of incubation (Fig. 1a). Moreover, enhancements in protease activity have been reported in response to application of rye grass residues (Nannipieri et al., 1983) as well as glucose and N application (Renella et al., 2007). Studies in pure cultures demonstrated that simple C and N sources including glucose, NH_4^+, and amino acids can inhibit the activity of proteases (Sharma and Singh, 2016). But there have been some controversial findings in soil and protease activity is dependent on the availability of C and N sources, (Geisseler and Horwath, 2008).

The products of enzymatic reactions expectedly serve as inhibitors with negative effects on reaction rate. L-arginine as an amino acid might be considered as a potential inhibitor of soil protease activity (Sharma and Singh, 2016). The present results, however, demonstrated that L-arginine acted as a stimulator, rather than an inhibitor, of general hydrolytic and protease activities. This was verified by positive responses of both indicators after the first 10 days of the incubation period (Figs. 1a and b).

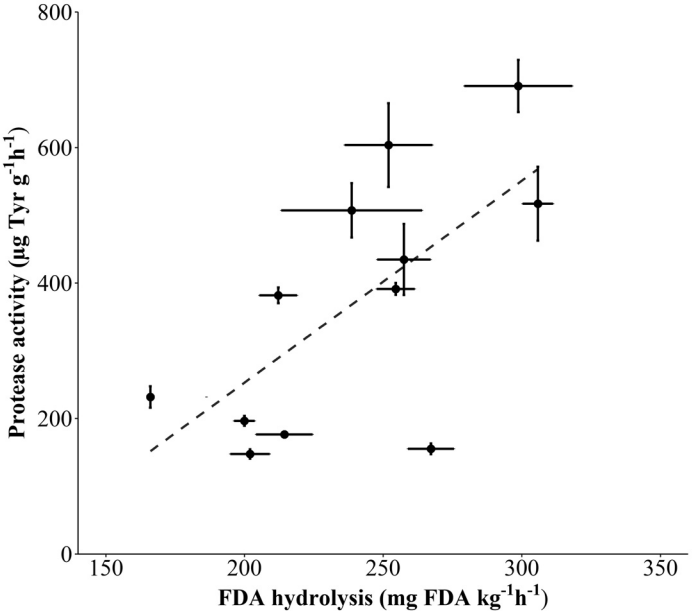

Fig. 2. Relationship between fluorescein diacetate (FDA) hydrolysis rate and protease activity during 40 days of incubation (r = 0.67, $P < 0.05$).

The correlation coefficient (r = 0.67, P < 0.05) revealed a significant relationship between the hydrolysis of fluorescein diacetate (FDA) and protease activity (Fig. 2). Similar results have been reported by Haynes (1999) and Perucci (1992). Soil organic C content has a positive influence on hydrolytic enzymes (Nourbakhsh, 2007). According to Haynes (1999), the organic and microbial composition of the soil was the main reason for the positive correlation between FDA hydrolysis and protease activity. Substrate induction is a key regulator of soil extracellular enzyme production. In spite of additional substrate, soil C and N availability can have a highly significant effect on hydrolytic enzyme regulation mechanisms (Geisseler and Horwath, 2009). The amino acids utilized as a simple and high-quality C source by the soil microbial community can be respired or appear in microbial biomass (McMahon and Schimel, 2017). The applied amino acids rapidly utilized by soil microorganisms less than 24 hours (Eilers et al., 2010). Then, hydrolytic enzymes produced by the new proliferating microbes increase as compensation mechanisms to prepare C sources from the complex substrate of soil (Sims and Wander, 2002; Allison and Vitousek, 2005). Therefore, FDA hydrolysis rate response to L-arginine confirmed that the amino acid may trigger all hydrolytic enzyme activities in soils indirectly.

4. Conclusion

Overall, the results clearly demonstrate that addition of L-arginine stimulated not only general hydrolytic activity but also protein hydrolysis in the forest soil tested. Thus, L-arginine seems to play a critical role in the rapid enhancement of soil protein hydrolysis capacity and it is anticipated that amino acids released in forest soils early during organic matter decomposition should boost soil protease activity.

Acknowledgement

The authors appreciate Isfahan University of Technology and the Soil Science Centre of Excellence for funding the project.

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