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RESEARCH ARTICLE

Effects of *Praxelis clematidea* invasion on soil nitrogen fractions and transformation rates in a tropical savanna

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Abstract Plant invasion has been reported to affect a mass of soil ecological processes and functions, although invasion effects are often context-, species- and ecosystem- specific. This study was conducted to explore potential impacts of *Praxelis clematidea* invasion on contents of total and available soil nitrogen (N) and microbial N transformations in a tropical savanna. Soil samples were collected from the surface and sub-surface layers in plots with non-, slight, or severe *P. clematidea* invasion in Hainan Province of southern China, which remains less studied, and analyzed for contents of the total and available N fractions and microbial N transformations. Results showed that total N content significantly increased in the surface soil but trended to decrease in the sub-surface soil in the invaded plots relative to the non-invaded control. Slight invasion significantly increased soil alkali-hydrolysable N content in the two soil layers. Soil net N mineralization rate was not significantly changed in both the soil layers, although soil microbial biomass N was significantly higher in plots with severe invasion than the control. There was no significant difference in content of soil N fractions between plots with slight and severe invasion. Our results suggest that invasion of *P. clematidea* promotes soil N accumulation in the surface soil layer, which is associated with increased microbial biomass N. However, the invasion-induced ecological impacts did not increase with further invasion. Significantly higher microbial biomass N was maintained in plots with severe invasion, implying that severe *P. clematidea* invasion may accelerate nutrient cycling in invaded ecosystems.

Keywords Plant invasion · Soil N stock · Microbial functioning · Plant and soil interactions · Global change

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Introduction

Exotic plant invasion can result in a mass of ecological impacts on invaded ecosystems (Jandova et al. 2014; van der Putten et al. 2007; Weidenhamer and Callaway 2010), mostly negative but sometimes positive (Marris 2009). It is most likely to alter the composition and functions of natural ecosystems (Dukes and Mooney 1999) and consequently cause environmental and economic problems (Callaway et al. 2004). Therefore, invasion ecology emerged as a highlighted research field and a deal of studies have been conducted to answer the three big questions proposed by the SCOPE program: which species invade, which habitats are invaded, and how can we manage invasion (Richardson and Pyšek 2006).

A lot of knowledge has been obtained to answer the first two questions. According to the Tens Rule, for instance, 650–10,400 species of alien vascular plant are potentially invasive plants around the world (Richardson and Pyšek 2006). Much attempt has been made to investigate distribution of invasive plant species in regions including the continental USA, the Australian Alps,

and China (Bradley et al. 2015; McDougall et al. 2005; Weber et al. 2008), and a list of useful databases (e.g., the Global Invasive Species Database; http://www.issg.org/database/welcome/) established to record invasive species at the global scale. Previous studies showed that habitats with rich resources and frequent disturbances are most likely to be invaded (Burke and Grime 1996), because resources such as light, water, and nutrients are requisites for plants to colonize a new habitat, and disturbances can provide opportunities for successful invasions (Seastedt and Pyšek 2011). Special growth strategies ensure invasive plants to outcompete their native competitors in invaded habitats (Callaway and Ridenour 2004; MacKown et al. 2009; Weidenhamer and Callaway 2010). The third question of invasive ecology, however, is difficult to answer based on the existing knowledge because the underlying mechanisms of a successful invasion remain incompletely understood (Hulme et al. 2013). Several hypotheses such as enemy releases and novel weapons have been proposed (Callaway and Ridenour 2004; Skurski et al. 2014; te Beest et al. 2009), but it is often hard to determine which mechanism(s) is predominant. More likely, predominant mechanisms underlying plant invasions depend greatly on the preexisting properties of ecosystems and invasive species and are various (Hulme et al. 2013; Sun et al. 2014).

Plant invasion can change lots of aboveground and belowground ecosystem processes and properties, but more attention has been paid on the visible aboveground ecosystems (van der Putten et al. 2007). In spite of linkages existing between aboveground and belowground components (Wardle et al. 2004), how plant invasion affects the belowground ecological processes and how the changed ecological processes feedback remain less studied (van der Putten et al. 2007). Compiling previous literature, we can find that contradictory observations exist for most of the investigated indices (e.g., soil carbon [C] and nitrogen [N] storage) and their underpinning stocks (e.g., soil C pool and available N) and flows (e.g., soil respiration and nitrification potential) (Sardans and Peñuelas 2012). This is likely attributable to the context-dependence nature of invasion effects on soil ecological processes (Hulme et al. 2013), which are driven by groups of functionally plastic but structurally robust microorganisms (Carey et al. 2015). As suggested by Hulme et al. (2013), further integrative measures of invasion effects on ecosystem stocks and flows would help improve our understanding on invasion impacts and accordingly make effective policies and decisions to manage and control invasion. Soil microbial community and nutrient cycling driven by microorganisms are susceptible to plant invasion (Souza-Alonso et al. 2014; Weidenhamer and Callaway 2010). Due to plant-soil feedbacks (Bardgett and van der Putten 2014; van der Putten et al. 2013; van der Putten et al. 2007), changed belowground ecosystem properties probably influence competitions between native and alien plant species (Kuebbing et al. 2014; Xiao et al. 2014).

Praxelis clematidea, a native species which distributes widely in South America (Wang et al. 2015; Waterhouse 2003), is an annual or short-lived perennial herb in the Asteraceae family. It is projected that *P. clematidea* could adapt and establish in diverse habitats, such as tropical rainforest and savanna, steppe, humid subtropics, marine west coast, and wet Mediterranean regions (USDA 2014). In fact, it has invaded in most of the aforementioned regions such as north Queensland, southern China, and Florida and could potentially affect native ecosystems and the environment (USDA 2014; Wang et al. 2015; Waterhouse 2003). As we know, however, ecological impacts of the invasive *P. clematidea* in new habitats have been rarely studied.

This case study was conducted to investigate invasion impacts of *P. clematidea* on the soil N fractions and microbial N transformations in a tropical savanna of southern China, a region which remains less studied relative to America and Europe which have been intensively studied (Hulme et al. 2013). A recent meta-analysis showed that plant invasion could increase soil available N concentration, regardless of life forms, functional groups of N fixation, or ecosystem types (Liao et al. 2008). Based on this observation, we hypothesized that *P. clematidea* invasion would also increase contents of the total and available soil N fractions in the studied savanna ecosystem (hypothesis I). Moreover, a previous modeling study suggested that invasion-induced ecological impacts were likely to increase with further invasion (Ogle et al. 2004). This trend was observed in temperate inland wetlands (Martina et al. 2014), but empirical studies remain lacking in tropical ecosystems (te Beest et al. 2015). According to these prediction and observations, we expected that in the studied savanna, the response magnitude of soil N fractions to *P. clematidea* invasion would increase as invasion severity increased (hypothesis II). Because soil N ammonification and nitrification processes depend greatly on soil N availability (Carey et al. 2015; Evans et al. 2001; Liao et al. 2008), we expected that microbial N transformations would also increase with increased soil N contents (hypothesis III). To prove the proposed hypotheses, multiple soil N fractions and microbial N transformation rates were analyzed to reveal the invasion-induced variations of soil N stocks and flows. Soil N fractions analyzed in this study include total N (TN), alkali-hydrolyzable N (AHN), water-soluble N, ammonium, and nitrate and could reveal responses of both total N and available N fractions to *P. clematidea* invasion.

Material and methods

Site descriptions

This study was conducted in Dongfang Datian National Natural Reserve (108°47′–108°49′E, 19°05′–19°17′N), which is reserved to protect *Cervus eldi hainanus* and therefore has experienced little anthropogenic disturbance since its

establishment in 1976. This region has a tropical monsoon climate, with obvious dry and wet seasons included in a year. The annual air temperature is 24.6 °C, with the lowest air temperature being 6.4 °C and the highest being 36.5 °C. The annual precipitation is 1019 mm but often occurs unevenly during a year, with more than 70% occurring during the wet season from July to October and the remaining occurring during the dry season. The annual evaporation reaches up to around 2000 mm. Consequently, a typical tropical savanna ecosystem presents in this reserve, with dominant species being several native herbs such as *Hyptis suavedens*, *Eragrostis zylindrica*, *Tridax procumbens*, *Borreria articularis*, and *Fimbristylis fusca*. In this savanna, alien species *P. clematidea* has invaded for years and therefore patches with different severities of *P. clematidea* invasion have been formed. In prior to invasion, the reserve had relatively homogenous soil because it has the same parent rock, experiences the same climate, and was covered by similar vegetation. This offers a good opportunity to explore the potential effects of *P. clematidea* invasion by using the space-for-time substitution method. At the study site, soil is classified as oxisol referring to the USDA soil taxonomy.

Sample collections

Fifteen quadrats with a size of 2 × 2 m^2 were established in the studied savanna: five were located in the non-invaded control plots, five in plots with slight invasion, and the other five in plots with severe invasion. The severity was defined as slight or severe invasion based on the predominance of *P. clematidea* (40 vs. >90% of total coverage) and invasion duration (6 vs. >10 years). In each quadrat, a 1 × 1 m^2 subplot was enclosed and bulk soils were collected from the surface (0–10 cm) and subsurface (10–20 cm) soil layers, after detectable plant residues and rocks were removed. Bulk soil, rather than rhizospheric soil, was chosen because the former can reflect relatively long-term invasion effect. Soil samples were sieved and mixed completely, and then separated into two parts for laboratory analyses. One part of each fresh soil sample was analyzed for soil moisture content, microbial biomass N, water-soluble N, ammonium, nitrate, and microbial N transformation rates immediately after being transported to lab; otherwise, soil samples were stored at 4 °C and analyzed within 2 weeks. The other one part of the soil samples was air-dried and sieved to determine soil pH, soil total N, and alkali-hydrolyzable N. Raw soils were also collected non-destructively to determine soil bulk density using the cutting-ring method.

Soil analyses

Soil moisture content, pH, bulk density, TN, ammonium, nitrate, and alkali-hydrolyzable N were assayed as described by Bao (2000). In brief, the soil moisture content was determined by oven-drying fresh soils at 105 °C for 24 h and weighting. Soil pH was tested using the potentiometry method (soil/water = 1:2.5) and a pH meter (PHS-3C, INESA Scientific Instrument Co. Ltd., Shanghai, China). Bulk density was determined by dividing soil volume by the according soil oven-dried weight. Soil TN content was determined using the Kjeldahl method. Moreover, fresh soil samples were extracted in 2 M KCl using a soil/solution ratio of 1:5. The extracts were then analyzed using colorimetry and ultraviolet spectrophotometry to determine the ammonium and nitrate contents, respectively. Alkali-hydrolyzable N was assayed using the alkaline-hydrolysis and diffusion method, i.e., the available N was reduced to NH_3 at 40 °C for 24 h after adding $FeSO_4$ powder and a NaOH solution, and then the NH_3 was absorbed using H_3BO_3 and titrated using H_2SO_4 to determine the AHN content.

Microbial biomass N content was determined using the CH_3Cl fumigation-K_2SO_4 extraction method as proposed by Wu et al. (1990). Briefly, two portions of 25 g of each fresh soil sample were weighted into containers: one container fumigated with CH_3Cl for 24 h while the other container un-fumigated as the control. After fumigation, 0.5 M K_2SO_4 solution was used to retain the extracted N, and then the supernatant solution was analyzed to determine N content by an automatic kieldahl apparatus (UDK 159, VELP Scientifica srl, Italy). Soil microbial biomass N content was calculated using an extraction coefficient of 0.54 (Brookes et al. 1985). Soil net N transformation rates were determined according to the method described by Stanford and Smith (1972). In details, 20 g of fresh soil was incubated at 25 °C for 1 month. During the incubation period, containers were weighted periodically and deionized water added to maintain soil moisture content. Before and after the incubation, soil ammonium and nitrate contents were assayed as described above. The differences in ammonium and nitrate contents of preincubated and postincubated soils were divided by incubation duration to indicate net microbial ammonification and nitrification rates, respectively. The sum of absolute ammonification and nitrification rates was considered as net N mineralization rate in this study.

Statistics

Two-way analysis of variance (ANOVA) was used to detect significant effects of invasion severity, soil layer, and interaction between both on environmental variables, content of soil N fractions and microbial properties. Because our main interest was to test the invasion effects on soil N fractions and microbial N transformation rates, one-way ANOVA with the Tukey HSD multiple comparisons was employed to compare means among plots with non-, slight, and severe invasion in each of the two soil layers when hypotheses of normality and homogeneity of variances were met. Otherwise, Welch test and the Games-Howell multiple comparisons were used. Bivariables Pearson correlation was employed to detect significant relationships between content of soil N fractions and environmental factors or microbial properties. Significance level was set at $p < 0.05$. All these statistics were conducted in IBM SPSS Statistics 22 (IBM Corp., New York,

Table 1 Soil physiochemical properties at surface (0–10 cm) and subsurface (10–20 cm) soil layers of control and invasion plots

Treatment	Soil layer	SM	BD	pH	SOC
Control	0–10 cm	0.12 ± 0.00	1.75 ± 0.02	4.23 ± 0.06	9.68 ± 0.39 a
	10–20 cm	0.13 ± 0.01	1.72 ± 0.01	4.20 ± 0.05	7.58 ± 0.27
	p value	0.393	0.189	0.784	0.002
Slight invasion	0–10 cm	0.09 ± 0.01	1.72 ± 0.06	4.04 ± 0.07	10.99 ± 0.53 a
	10–20 cm	0.12 ± 0.02	1.81 ± 0.05	4.02 ± 0.08	7.16 ± 0.72
	p value	0.123	0.291	0.862	0.003
Severe invasion	0–10 cm	0.13 ± 0.03	1.75 ± 0.02	4.29 ± 0.08	14.20 ± 0.51 b
	10–20 cm	0.14 ± 0.01	1.78 ± 0.03	4.30 ± 0.07	9.18 ± 0.97
	p value	0.808	0.381	0.941	0.002

Differences among treatments are not significant at $p < 0.05$ level except for SOC content at surface soils. Statistical *p* value indicates difference significance of those indexes between the two soil layers under each treatment for each tested index. Numbers in the cells are means followed by standard errors ($n = 5$ for the others except $n = 3$ for bulk density)

SM soil moisture (g g^{-1}), *BD* bulk density (g cm^{-3}), *SOC* soil organic carbon (g kg^{-1})

US), and graphs were made in SigmaPlot 10.0 (Systat Software Inc., California, USA).

Results

Effects of *P. clematidea* invasion on soil physiochemical properties

Our results showed that *P. clematidea* invasion did not significantly change soil physiochemical properties including soil moisture content, bulk density, and pH in both of the surface and subsurface soil layers ($p > 0.05$, Table 1). Relative to the non-invasion control, *P. clematidea* invasion significantly increased soil organic carbon (SOC) content in the surface soil ($p < 0.001$) but did not significantly alter SOC content in the subsurface soil layer ($p = 0.152$, Table 1). Regardless in the control or plots with *P. clematidea* invasion, SOC content was significantly higher in the surface soil than in the subsurface soil ($p < 0.05$). However, the other soil physiochemical properties investigated in this study, including soil moisture content, bulk density, and pH value, were not significantly different between the two soil layers ($p > 0.05$, Table 1).

Effects of *P. clematidea* invasion on soil N fractions and transformations

Two-way ANOVA showed that invasion significantly increased soil alkali-hydrolyzable N and microbial biomass N contents ($p < 0.05$, Table 2). All the investigated soil N indices

Table 2 Results of two-way ANOVA on soil nitrogen (N) fractions and microbial N transformation rates

	Invasion severity		Soil layer		Interaction	
	F	*p*	*F*	*p*	*F*	*p*
TN	1.436	0.258	16.052	0.001	4.106	0.029
AHN	11.453	<0.001	30.529	<0.001	1.018	0.376
WSN	2.246	0.128	20.353	<0.001	1.422	0.261
Ammonium	0.354	0.706	57.931	<0.001	1.048	0.366
Nitrate	0.701	0.506	26.285	<0.001	2.102	0.144
MBN	10.105	0.001	59.168	<0.001	2.214	0.131
NA	1.344	0.280	15.280	0.001	1.471	0.250
NN	0.245	0.784	28.287	<0.001	0.704	0.504
NM	0.328	0.723	43.006	<0.001	0.993	0.385

Numbers presented in cells are statistical *F* value and significance level *p*

TN soil total N, *AHN* alkali-hydrolyzable N, *WSN* water-soluble N, *MBN* microbial biomass N, *NA* net ammonification rate, *NN* net nitrification rate, *NM* net mineralization rate

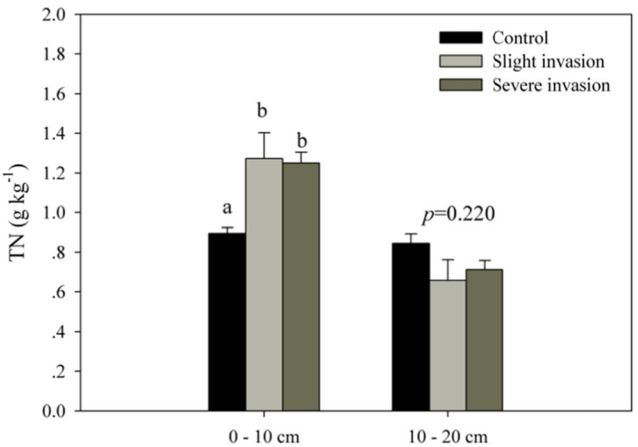

Fig. 1 Content of total nitrogen (TN) at surface (0–10 cm) and subsurface (10–20 cm) soil layers of control and invasion plots. *Bars* indicate means with error bars being standard errors ($n = 5$). Different *lowercase letters* indicate significant differences at $p < 0.05$ level among treatments at each soil layer. Statistical *p* value is listed above the bars in case differences among treatments are not significant

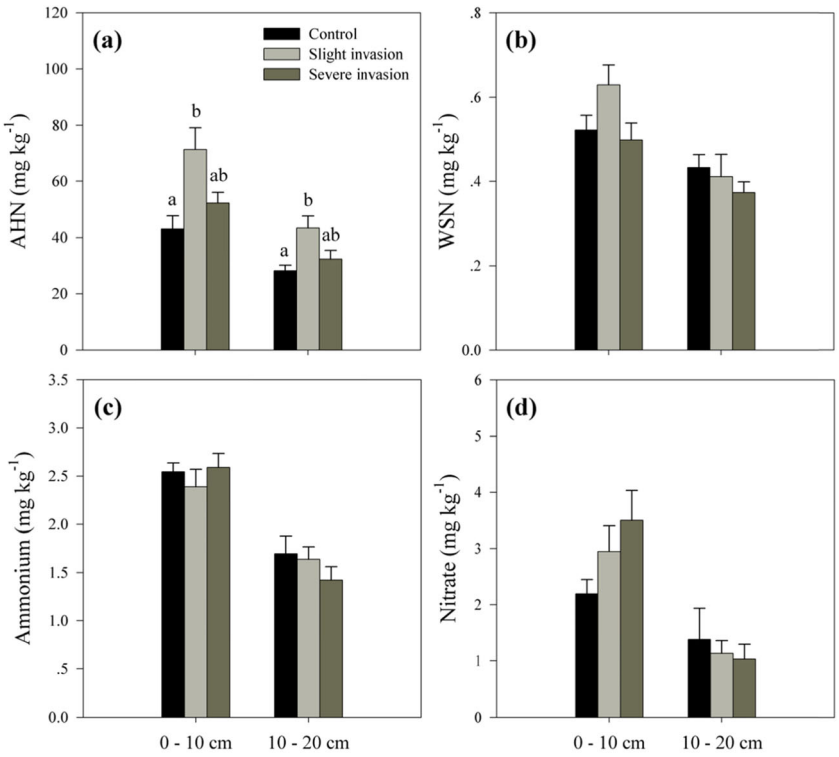

Fig. 2 Content of alkali-hydrolyzable nitrogen (*AHN*; **a**), water-soluble nitrogen (*WSN*; **b**), ammonium (**c**), and nitrate (**d**) at surface (0–10 cm) and subsurface (10–20 cm) soil layers of control and invasion plots. *Bars* indicate means with error bars being standard errors ($n = 5$). Different *lowercase letters* indicate significant differences at $p < 0.05$ level among treatments at each soil layer

were significantly different between the two soil layers ($p < 0.05$, Table 2). For soil TN content, interaction between invasion and soil depth was statistically significant ($p = 0.029$), but the interactive effects between the two factors were not significant for the other soil N indices ($p > 0.05$, Table 2).

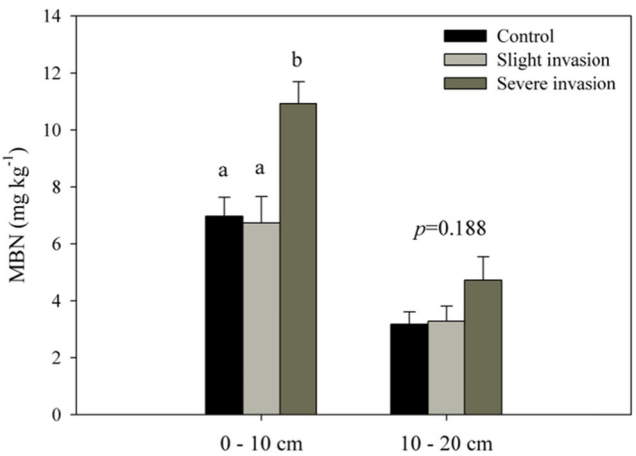

Fig. 3 Content of microbial biomass nitrogen (*MBN*) at surface (0–10 cm) and subsurface (10–20 cm) soil layers of control and invasion plots. *Bars* indicate means with error bars being standard errors ($n = 5$). Different *lowercase letters* indicate significant differences at $p < 0.05$ level among treatments at each soil layer. Statistical p value is listed above the bars in case differences among treatments are not significant

As shown in Fig. 1, one-way ANOVA indicated that soil TN content was significantly higher in the invaded plots than in the control in the surface layer ($p = 0.012$), being 1.27, 1.25, and 0.90 g kg^{-1}, respectively. In the subsurface soil, however, only a minor and statistically insignificant difference was observed for soil TN content among the control and plots with slight and severe invasion ($p = 0.220$, Fig. 1). Likewise, invasion increased soil AHN content in both the soil layers (Fig. 2a). Slight invasion significantly increased soil AHN content in the two soil layers ($p < 0.05$, Fig. 2a). Severe invasion tended to increase the AHN content but the increase was not statistically significant ($p > 0.05$, Fig. 2a). Moreover, *P. clematidea* invasion did not significantly change soil ammonium, nitrate, and water-soluble N contents in the two soil layers ($p > 0.05$, Fig. 2b–d).

In the present study, microbial biomass N and N transformation rates were analyzed to indicate microbial activity. Results showed that severe invasion significantly increased soil MBN content in the surface soil ($p = 0.005$) and tended to increase it in the subsurface soil ($p = 0.188$, Fig. 3). Relative to the control, slight invasion did not significantly change MBN content in both the soil layers ($p > 0.05$, Fig. 3). Although soil MBN content increased, invasion did not cause any significant change of net ammonification rate, net nitrification rate, or net mineralization rate in the surface soil ($p > 0.05$ for all, Fig. 4). In the subsurface soil, *P. clematidea* invasion tended to increase net ammonification rate, net nitrification rate, and net N mineralization rate

relative to the control ($p > 0.05$, Fig. 4). In this study, net ammonification rate was negative under all the treatments and an order of magnitude lower than net nitrification rate (comparing Fig. 4a, b).

Correlations between soil N fractions and physiochemical or microbial properties

Soil N fractions did not show significant correlations with the tested environmental variables such as soil moisture content, pH, and bulk density ($p > 0.05$). The only exception was soil alkali-hydrolyzable N which was significantly related to soil pH ($p = 0.025$, Table 3). Unlike environmental variables, microbial properties showed significant correlations with soil N fractions. In particular, microbial biomass N was significantly positively related to soil total and available N fractions ($p < 0.05$), with an exception of water soluble N ($p = 0.102$, Table 3). Soil net nitrification and mineralization rates also showed significantly positive correlations with soil N fractions ($p < 0.01$). However, net soil ammonification rate was not significantly related to soil N contents ($p > 0.05$ for all, Table 3).

Discussion

As expected, total N content in the surface soil significantly increased in plots with *P. clematidea* invasion relative to the control, which partially supports our hypothesis I. This is consistent with the observations in most previous studies (Liao et al. 2008; Martina et al. 2014; Wolkovich et al. 2010), although negative or neutral effect has been ever reported (Carey et al. 2015; Ehrenfeld 2003). This increase in plots with invasion of *P. clematidea*, a non N-fixing species (USDA 2014), may be ascribed to three important underground ecological processes (Liao et al. 2008). First, invasive plants could have relatively higher biomass than do native species and therefore are able to increase C input into soil, which might offer soil microorganisms more C substrate to fix N in the soil (Hooper et al. 2000; Knops et al. 2002; Luo et al. 2006). Although community productivity in the studied plots was not investigated, we observed that *P. clematidea* individuals were obviously higher than native grass species. This implies that the invaded plots may have higher community productivity than the control. As observed in previous studies, ecosystem C and N stocks were significantly related to community productivity and litter mass (Martina et al. 2014).

Second, soil TN increase could be associated with increased microbial biomass N in the invaded plots (Table 3), because high microbial biomass N content benefits soil N accumulation (Ehrenfeld 2003; Knops et al. 2002). Understanding on this associated change in the two N pools

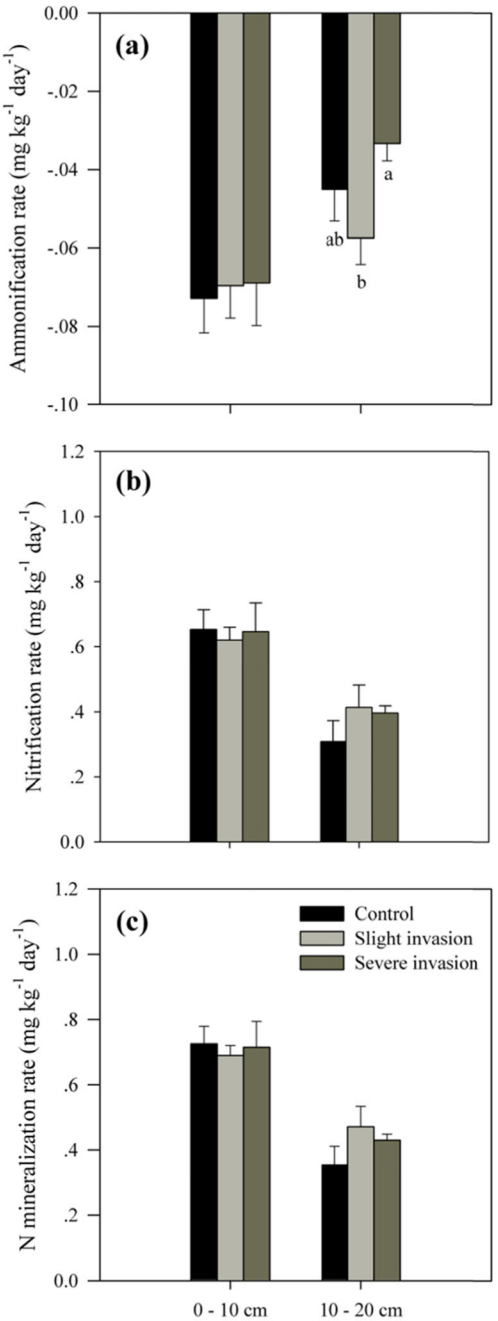

Fig. 4 Ammonification rate (**a**), nitrification rate (**b**), and nitrogen mineralization rate (**c**) at surface (0–10 cm) and subsurface (10–20 cm) soil layers of control and invasion plots. *Bars* indicate means with error bars being standard errors ($n = 5$). Different *lowercase letters* indicate significant differences at $p < 0.05$ level among treatments at each soil layer

remains incomplete, but this is likely explained by the fact that soil organic matter which contain and store N are mainly derived from microbial products (Cotrufo et al. 2013). Furthermore, soil microorganisms can assimilate N to maintain their growth and reproduction and could thus promote soil N accumulation by increasing microbial structural and metabolism products (Burger and Jackson 2003). Therefore,

Table 3 Correlations between content of soil nitrogen fractions and environmental or microbial properties

	SM	BD	pH	MBN	NA	NN	NM
TN	−0.038	−0.049	−0.207	0.637**	−0.220	0.597**	0.620**
WSN	−0.291	−0.441	−0.293	0.305	−0.308	0.539**	0.577**
AHN	−0.090	0.135	−0.409*	0.500**	−0.310	0.590**	0.622**
Ammonium	0.008	−0.156	−0.192	0.574**	−0.260	0.818**	0.843**
Nitrate	0.026	−0.188	−0.171	0.627**	−0.169	0.679**	0.699**

Numbers in the cells are correlation coefficients

TN total nitrogen, *WSN* water-soluble nitrogen, *AHN* alkali-hydrolyzable nitrogen, *SM* soil moisture, *BD* bulk density, *MBN* microbial biomass nitrogen, *NA* net ammonification rate, *NN* net nitrification rate, *NM* net mineralization rate

*$p < 0.05$; **$p < 0.01$

microbial biomass N showed a significantly positive relationship with soil total N in previous and the present studies. Third, relatively developed root system could offer invasive species greater opportunity to utilize N-contained compounds which is stored in the subsoil and cannot be reached by native plants (Liao et al. 2008; Luo et al. 2006). This is indirectly supported by the observation in this study that soil TN content decreased in the subsurface soil in plots with *P. clematidea* invasion relative to the control. Comparing the invaded plots with the control, however, soil TN increments in the surface soil were greater than the decrements in the subsurface soil. This results in mutually contradictory statistical outputs that invasion effect on soil TN content was significant in the surface soil when one-way ANOVA was employed but was not significant when two-way ANOVA was conducted, suggesting that invasion effects vary as soil depth changes (also refer to significant interactions for TN between invasion and soil depth in Table 2). This pattern could be attributed to different tradeoffs between N inputs and outputs in the two soil layers under *P. clematidea* invasion, with relatively higher N inputs (by litter fall and root exudates) in the surface soil than in the subsurface soil. Despite remaining incompletely understood, this observation implies that changes of soil N content cannot be fully explained by differences of root system between the invasive and native species. Direct evidence can be provided by comparing distribution zone and activity of plant roots between the invaded and control plots, which was not measured in the present study.

In this study, multiple soil available N fractions, i.e., alkali-hydrolyzable N, water soluble N, ammonium, and nitrate, were analyzed to detect potential changes in soil available and easily utilizable N content, because they are sensitive to environment changes including plant invasion (Liao et al. 2008; Roberts et al. 2009; Souza-Alonso et al. 2014). Opposite to our expectation, however, only soil alkali-hydrolyzable N content was significantly changed by *P. clematidea* invasion. The other labile N fractions investigated in this study were not significantly altered in the two soil layers. Therefore, our hypothesis II is objected by our observations that soil total and available N contents do not further increase with increased invasion severity.

Although plant invasion may positively affect soil ammonium and nitrate contents (Liao et al. 2008; Souza-Alonso et al. 2014), negative or neutral effect has also been reported in previous studies (Christian and Wilson 1999; Ehrenfeld 2003). This discrepancy may be attributable to the site or species dependence of invasion effects (Christian and Wilson 1999; MacKown et al. 2009). From the perspective of nutrient utilization, for instance, invasive species which can uptake N rapidly would be easy to invade successfully in N-rich ecosystems while those which can stimulate production of available N would easily invade in N-poor ecosystems. In this study, soil ammonium and nitrate contents were not affected by *P. clematidea* invasion. This is mutually supported with the observation that net ammonification and net nitrification rates were comparable in the control and invaded plots in this study, because ammonium and nitrate are either product or substrate for the two important N processes in the soil.

However, *P. clematidea* invasion increased soil alkali-hydrolyzable N content. As what is known, alkali-hydrolyzable N constitutes of diverse easily utilizable N-contained compounds, such as inorganic ammonium and nitrate, easily hydrolyzable proteins, and several amino sugars (Bao 2000; Roberts et al. 2009). This increase of alkali-hydrolyzable N content was statistically significant at the early stage of invasion but became insignificant at the late stage of invasion, supporting the idea that invasion effects are very likely non-linear and varied with the development of invasion (Christian and Wilson 1999). Nevertheless, inorganic soil N content and N mineralization rates were not changed in plots with invasion relative to the control, implying that *P. clematidea* invasion may affect the easily utilizable organic N materials rather than inorganic N compounds (Knops et al. 2002). Although inorganic N (i.e., ammonium and nitrate) are generally considered preferably utilizable to plants (Christian and Wilson 1999; Ehrenfeld 2003; Sardans and Peñuelas 2012), information could be missed if easily utilizable organic N compounds are overlooked. Relative to soil ammonium and

nitrate contents, our results suggest that soil alkali-hydrolyzable N could better indicate soil available N content and be more highly sensitive to environmental changes.

Previous studies reported plant invasion can increase soil microbial biomass (Liao et al. 2008). Consistent with the observation, we found that *P. clematidea* invasion increased soil microbial biomass N in this study. Soil containing higher microbial biomass could maintain faster nutrient cycling when all soil microorganisms are in active state. In the present study, net ammonification rate was negative, regardless of soil layer or vegetation condition. This indicates that ammonium production rate in the studied soil is lower than ammonium consumption rate, therefore generating negative net ammonification rate (Turner et al. 2007). Yet, ammonium depletion did not result in a statistically significant decrease of soil ammonium content. Moreover, invasion did not significantly change net microbial N transformation rates investigated in this study. The lack of changes in net microbial N transformation rates could be ascribed to the offset between mineral N production and consumption processes. For instance, increased soil total and available N contents could accelerate microbial N mineralization (Masunga et al. 2016). However, higher microbial biomass N in the invaded plots could have increased microbial N assimilation (Burger and Jackson 2003), therefore consuming more soil mineral N and lowering microbial N mineralization rate. Finally, the invasion-induced changes in net microbial N transformation rates depend greatly on the balance between these mineral N production and consumption processes. Significant changes cannot be detected in this study, probably due to the lack of investigations on the rates of mineral N production and consumption in different processes correspondingly. Further studies, e.g., using stable isotope technique to determine gross N transformation rates, would improve our understanding on the invasion-induced changes in soil N transformations.

In spite of non-significant invasion effect, net nitrification and net mineralization rates were significantly related with soil N contents, an observation supporting our hypothesis III and consistent with previous studies (Masunga et al. 2016). This could be attributable to the fact that soil N-contained compounds are substrates or products in the soil N processes mentioned above (Carey et al. 2015; Knops et al. 2002). It is rational that the N transformation rates depend on or determine the concentrations of substrates or products involved in the processes, although these differences originated mainly from the different soil layers but not from invasion effects.

Conclusions

Invasion of *P. clematidea* significantly increased soil total N content in the surface soil and tended to decrease soil TN in the subsurface soil in this tropical savanna. Soil alkali-hydrolyzable N content, rather than the inorganic ammonium or nitrate concentration, was significantly higher in the invaded plots than in the control, implying that the easily utilizable organic N-contained compounds could have increased due to *P. clematidea* invasion. These results indicate that plant invasion can maintain higher community biomass which may promote soil N stock within a certain time scale. Moreover, *P. clematidea* invasion increased soil microbial biomass. The consistent trend of soil N stock and microbial biomass N could be due to the fact that microbial products account for a great proportion of the stored soil organic matter. On the other hand, the increased soil microbial biomass induced by *P. clematidea* invasion indicates that invasion could accelerate nutrient cycling in the invaded ecosystems when soil microorganisms are in active state and soil microbial community functions do not become less efficient. Our results suggest that great uncertainties exist when to predict soil C and N dynamics under plant invasion. More studies, e.g., combining with further plant-soil interactions and assays of soil microbial communities and functional genes involving in soil C and N cycling, could help us further understand the ecological impacts of plant invasion.

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