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RESEARCH ARTICLE

Using golden apple snail to mitigate its invasion and improve soil quality: a biocontrol approach

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Abstract

The invasive and widespread golden apple snail (GAS, *Pomacea canaliculata*) is a harmful crop pest in many parts of Asia. The heavy use of molluscicides to control GAS could result in soil and water pollution as well as in loss of biodiversity. A sustainable and pollution-free control method is urgently needed to counteract this invasion. In this study, we proposed using dried and powdered GAS residue to neutralize and fertilize soils. We compared the effects of adding GAS residue (i.e., ground GAS shell and meat residue) to the effects of adding lime upon soil properties and microbes in a greenhouse pot experiment. Each pot was incubated for 120 days, and soil pH, nutrients, microbial species, and enzyme activity were assessed. Results showed that addition of GAS residue significantly improved soil pH, contents of total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and available nitrogen but decreased soil available phosphorus (AP) content due to phosphorus sorption induced by soil organic matter (OM) and high pH. The GAS residue added to soil released nutrients and alleviated soil acidity, as well as provided more resources to soil microbes to increase their bioactivity, although lime addition was better at mitigating soil acidity. We found that with added GAS residue of 25 g kg^{-1}, the soil nitrate nitrogen (NO_3-N) content increased by 10 times; microbial biomass increased by 43%; and enzyme activity of β-1,4-glucosidase, β-1,4-*N*-acetylglucosaminidase, and β-D-cellobiosidase also were enhanced, compared to the control. Our findings suggest that GAS residue functions well as a fertilizer and soil amendment to aid the remediation of barren and acidic soils, making it a valuable and useful option in the control of the invasive GAS.

Keywords Biocontrol · Golden apple snail · Microbial community · Soil nutrients · Soil pH · Waste management

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Abbreviations

NH_4-N	Ammonium nitrogen
NO_3-N	Nitrate nitrogen
TP	Total phosphorus
TOC	Total organic carbon
TN	Total nitrogen
AP	Available phosphorus
G+	Gram-positive bacteria
G−	Gram-negative bacteria
A	Actinomycetes
B	Bacteria (the sum of G+ and G−)
F	Fungi
BG	β-1,4-Glucosidase
CB	β-D-Cellobiosidase
NAG	β-1,4-*N*-acetylglucosaminidase
ACP	Acid phosphatase

Introduction

Golden apple snail (GAS, *Pomacea canaliculata*) was initially introduced from Argentina to Taiwan (China) in 1979 as a high-protein food for humans (Mochida, 1991). However, this distasteful snail was not commercially successful and was often discarded into backyards and then dispersed into rivers or streams. Since then, GAS has become invasive, spreading into rice fields throughout Southeast Asia (Carlsson et al., 2004; Chiu et al., 2014), as well as rice fields in Florida, Louisiana, and Mississippi, USA (Olivier et al., 2016; Gooding et al., 2018). In recent years, GAS has increased its distribution, invading agricultural and natural ecosystems. Traditional control methods are not effective (Halwart, 1994), so a cost-effective and environmentally friendly strategy to control GAS is urgently needed (Halwart, 1994).

Currently, the most commonly used control method is the application of molluscicides (e.g., niclosamide and metaldehyde) (Palis et al., 1997; Attademo et al., 2016), which are widely used throughout Southeast Asia (San Martín et al., 2008; Olivier et al., 2016). Although these chemical methods could cause 70–100% mortality of GAS at 10 ppm, they have adverse environmental impacts and are expensive for farmers (Huang et al., 2003; Castle et al., 2017). For example, the molluscicides used in the Philippines are highly harmful and toxic to rice seedlings and some aquatic organisms, as well as human skin, at the concentrations needed to kill GAS (Takougang et al., 2007; Joshi et al., 2008; Hallett et al., 2016). Metaldehyde, which is safe for fish and other aquatic life, has been applied to control GAS (Calumpang et al., 1995; Ramdwar et al., 2018), but requires a water temperature above 25 °C to be effective (Litsinger and Estano, 1993). Although the application of molluscicides helps protect crops damaged by GAS, the amount of dead GAS in paddy fields can cause serious environmental problems, such as water and air pollution (Pimentel et al., 2005; Olivier et al., 2016; Ibrahim et al., 2017). Therefore, the management of GAS waste also requires further research. Some research has evaluated the beneficial effects of oyster shells used as an alkaline lime on chemical and biological properties of the soil that has been acidified and degraded due to the acid rain and/or the over-use of nitrogen (N) fertilizers (Lee et al., 2008). However, whether GAS residue could be used as a liming material for soil improvement remains unknown.

Soil acidification is becoming increasingly serious throughout the world, largely due to intensive agriculture and over-use of N fertilizers (Van Breemen et al., 1982; Sumner and Noble, 2003; Guo et al., 2010; Smith et al., 2016). The application of each 50 kg ha^{-1} of ammonium nitrogen (NH_4-N) produces about 4 kmol of H^+ ha^{-1} $year^{-1}$ and requires about 500 kg $CaCO_3$ ha^{-1} $year^{-1}$ to neutralize it (Goulding and Annis, 1998). Although the use of lime can increase soil pH, long-term application of lime could lead to soil compaction and a decrease in the availability of Si and P (Kumar et al., 2007; Haynes and Zhou, 2018). These potential changes to soil properties, such as soil pH, bulk density, and the availability of Si and P, not only impact crop growth, but also influence the community composition and activity of soil microbes (Smith et al., 2002; Pietri and Brookes, 2008; Schlich and Hund-Rinke, 2015; Boot et al., 2016).

Microbial communities and activities are sensitive to changes in soil properties such as soil nutrient availability including NH_4-N, NO_3-N, TN, moisture content, and pH (Schipanski and Drinkwater, 2012; Bowles et al., 2014). White et al. (1979) and Bååth and Anderson (2003) reported that phospholipids fatty acids (PLFAs) were good indicators for estimating soil microbial community composition and microbial biomass. Extracellular enzymes such as β-1,4-glucosidase (BG), acid phosphatase (ACP), β-1,4-*N*-acetylglucosaminidase (NAG), and β-D-cellobiosidase (CB) are commonly used to estimate the cycling of soil nutrients and carbon (Allison and Vitousek, 2005; Geisseler and Horwath, 2009). However, the effects of GAS residue on microbial composition and enzyme activity are largely unknown.

In this study, we evaluated the performance of GAS residue as a fertilizer. GAS are inactive and unable to move when their home field is drained, and the shells are fragile, especially in dry conditions (Litsinger and Estano, 1993). In these conditions, adding ground-up GAS residue to soils and then mixing it thoroughly with soils for use as a fertilizer may be possible. In addition, this cost-effective and readily available resource should encourage farmers to collect and use snails, thereby helping to control the invasive GAS spread.

We hypothesized that application of powdered snail residue (i.e., a mixture of snail meat and shell) to non-irrigated farmland plowed into the soil would release nutrients and alleviate soil acidity, as well as providing more resources to soil microbes to increase their bioactivity. In this study, we aimed to (1) evaluate the effects of different GAS residue concentrations on soil pH, carbon stocks, nutrients (N and P), microbial community composition, and enzyme activity; (2) explore the relationships among these soil properties after the application of GAS residue; and (3) test what differences between GAS residue and lime application to improve soil nutrient availability and decrease soil acidity.

Materials and methods

Soil and snail

Soil and GAS used in this study were collected from paddy fields located at the Teaching and Research Farm (23° 14′ 22″ N, 113° 37′ 57″ E), South China Agricultural University, Guangzhou, China. About 600 GAS were collected at the

initial inundated stage (April 22, 2018) of rice growing, and then flushed with tap water, followed by deionized water to clean the dirt off the surface of the shell. The GAS was frozen at − 40 °C for 24 h. After that, the dead GAS was oven-dried, crushed, ground, and stored in a desiccator. The soil was collected after the rice harvest and transported to the laboratory, aired for 2 h, crumbled by hand and a rubber hammer, homogenized thoroughly, and passed through a 2-mm mesh to remove rocks, roots, and organic residue and then air-dried. According to the USDA textural soil classification, the soil was classified as sandy clay, consisting of medium (37%) and fine (23%) sand, silt (5%), and clay (35%). The soil and GAS residue properties were measured before the experimental incubation to obtain baseline values. The soil had pH of 6.34, TOC of 16.38 g kg^{-1}, TN of 2.20 g kg^{-1}, and TP of 0.38 g kg^{-1} (Table 1). The GAS residue added to the soil had 23.02% C, 0.58% N, and 0.46% P contents.

Experimental setup

Greenhouse incubation experiments were conducted on the campus of South China Agricultural University. The following three treatments were used (Fig. 1): (1) the control (CK, only soil was used), (2) GAS residue treatment (SR, with GAS residue added to the soil), (3) lime treatment (SL, with lime added to the soil). The two treatments had six levels (0.5, 1, 2.5, 25, 50, and 100 g kg^{-1} of snail residue or lime added) with five replicates. The 100 g kg^{-1} level treatment was used to evaluate the risk of over-use of GAS residue or lime. For the control, 2 kg of air-dried soil was packed into a plastic pot (height = 260 mm, a top diameter = 200 mm, a bottom diameter = 180 mm) with a couple of tiny holes at the bottom. The pot, with a piece of filter paper beneath, was placed on a plastic dish to prevent the loss of soil and GAS residue. For the SR and SL treatments, a 2-kg mix of the air-dried soil and treatment addition were used to pack the pots. At the beginning of the incubation, about 600–800 mL of deionized water was used to each pot. During the incubation period, 400 mL of deionized water per week (watered every 3 days) was sprayed on the surface of each pot to keep the soil moisture content (about 300–400 g kg^{-1}) sufficient to meet the needs of microbes. The average temperature in the greenhouse was maintained at 25 °C. The incubation process lasted 4 months. During the incubation period,

Table 1 Total organic carbon (TOC) and soil nutrients including nitrate nitrogen (NO_3-N), total nitrogen (TN), ammonium nitrogen (NH_4-N), total phosphorus (TP), and available phosphorus (AP) at different addition levels of the snail residue (SR) and lime (SL) treatments

	Treatment*	TOC (g kg^{-1})	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	NH_4-N/NO_3-N	TN (g kg^{-1})	TP (g kg^{-1})	AP (mg kg^{-1})
Background	CK	16.38 ± 0.56A	4.20 ± 0.26A	2.21 ± 0.09A	0.52 ± 0.01A	2.20 ± 0.07A	0.38 ± 0.01A	30 ± 0.05A
	SR0.5	16.35 ± 0.43A	4.21 ± 0.23A	2.19 ± 0.12A	0.52 ± 0.01A	2.20 ± 0.07A	0.40 ± 0.02A	29 ± 0.02A
	SR1	16.37 ± 0.48A	4.22 ± 0.36A	2.18 ± 0.14A	0.52 ± 0.01A	2.19 ± 0.06A	0.36 ± 0.02A	29 ± 0.01A
	SR2.5	16.29 ± 0.32A	4.19 ± 0.42A	2.19 ± 0.13A	0.52 ± 0.02A	2.21 ± 0.08A	0.39 ± 0.03A	29 ± 0.02A
	SR25	16.33 ± 0.26A	4.19 ± 0.41A	2.19 ± 0.14A	0.52 ± 0.01A	2.20 ± 0.08A	0.39 ± 0.02A	28 ± 0.01A
	SR50	16.33 ± 0.53A	4.18 ± 0.24A	2.20 ± 0.07A	0.53 ± 0.01A	2.22 ± 0.11A	0.40 ± 0.01A	28 ± 0.03A
	SR100	16.36 ± 0.41A	4.20 ± 0.22A	2.19 ± 0.11A	0.52 ± 0.01A	2.20 ± 0.13A	0.38 ± 0.01A	29 ± 0.02A
	SL0.5	16.55 ± 0.61A	4.21 ± 0.23A	2.20 ± 0.12A	0.52 ± 0.02A	2.18 ± 0.14A	0.37 ± 0.02A	29 ± 0.02A
	SL1	16.46 ± 0.36A	4.21 ± 0.30A	2.22 ± 0.11A	0.53 ± 0.02A	2.21 ± 0.11A	0.36 ± 0.02A	30 ± 0.04A
	SL2.5	16.32 ± 0.38A	4.20 ± 0.33A	2.19 ± 0.09A	0.52 ± 0.01A	2.20 ± 0.07A	0.37 ± 0.02A	29 ± 0.03A
After treatment	CK	16.04 ± 0.66e	3.78 ± 0.43de	1.82 ± 0.08e	0.49 ± 0.04ab	2.19 ± 0.16c	0.40 ± 0.03d	26 ± 0.01ab
	SR0.5	17.56 ± 0.9cde	5.51 ± 1.38de	2.15 ± 0.15cd	0.45 ± 0.13bc	2.44 ± 0.16bcd	0.43 ± 0.04bcd	28 ± 0.00ab
	SR1	17.57 ± 0.62cde	8.18 ± 0.50c	2.26 ± 0.09bc	0.28 ± 0.02cd	2.40 ± 0.10cd	0.53 ± 0.05cd	27 ± 0.02ab
	SR2.5	18.46 ± 0.12bc	12.33 ± 6.06bc	2.07 ± 0.28cde	0.21 ± 0.01de	2.32 ± 0.13cd	0.51 ± 0.00bc	27 ± 0.02ab
	SR25	26.41 ± 4.43ab	38.29 ± 0.94a	2.97 ± 0.06ab	0.08 ± 0.00f	5.08 ± 0.42a	0.55 ± 0.07bcd	31 ± 0.06ab
	SR50	27.96 ± 0.31a	30.04 ± 1.03ab	3.40 ± 0.25a	0.11 ± 0.01ef	4.87 ± 0.17a	0.59 ± 0.02bc	29 ± 0.06ab
	SR100	37.57 ± 2.53a	30.17 ± 1.85ab	4.86 ± 0.58a	0.16 ± 0.02ef	3.84 ± 0.13ab	0.80 ± 0.07a	25 ± 0.04b
	SL0.5	17.79 ± 0.26cde	4.40 ± 0.66de	2.03 ± 0.07cde	0.49 ± 0.10ab	2.52 ± 0.07bc	0.40 ± 0.03d	29 ± 0.02ab
	SL1	16.28 ± 0.78de	5.33 ± 0.45d	1.92 ± 0.06de	0.37 ± 0.04bc	2.11 ± 0.08d	0.47 ± 0.08bcd	31 ± 0.01a
	SL2.5	18.21 ± 0.66cd	2.89 ± 0.52e	2.11 ± 0.08cd	0.80 ± 0.19a	2.27 ± 0.20cd	0.43 ± 0.03cd	33 ± 0.03a

*CK indicates the control; SR indicates the addition of GAS residues; SL indicates the addition of lime; the numbers at the end of SR and SL indicate the addition levels. The results are presented as mean ± SE, the different capital letters in each column represent the significance of background values among different treatments, while the different lowercase letters in each column suggest the significance of treated soils between different treatments according to Duncan's multiple range test

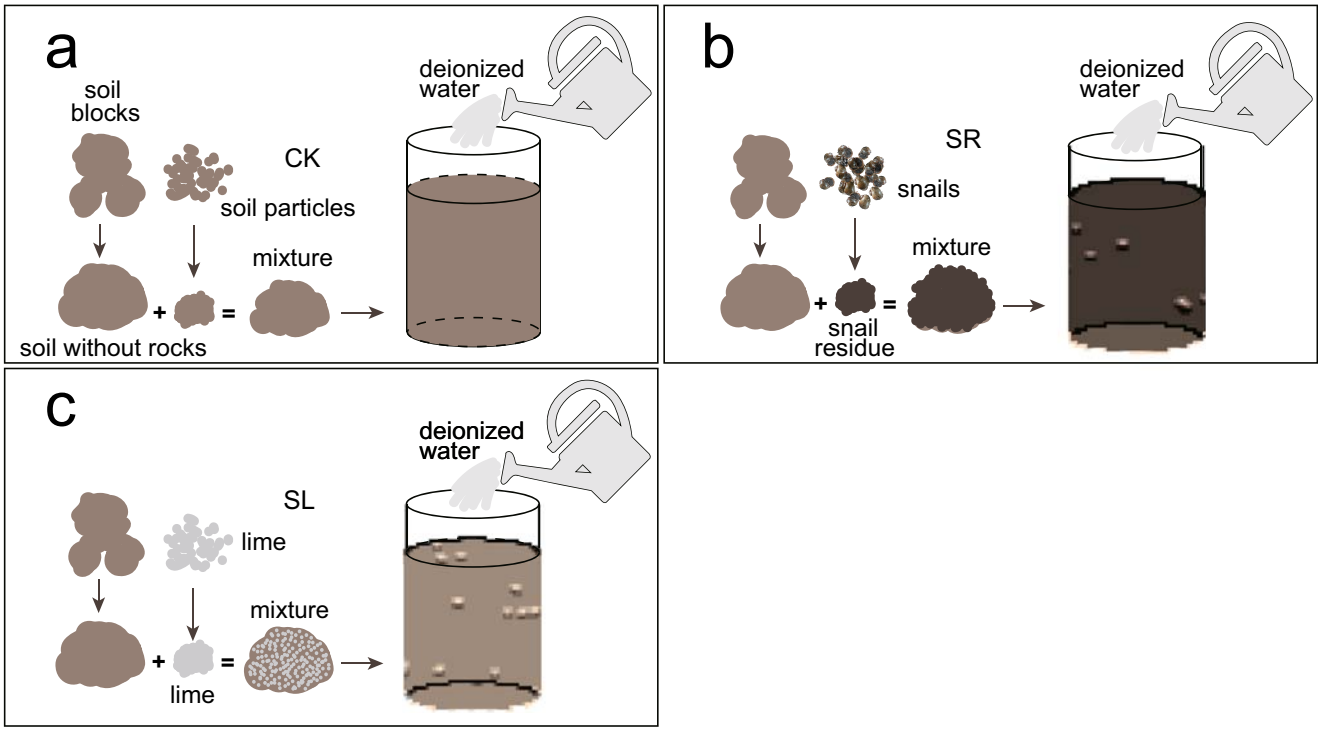

Fig. 1 Schematic representation of the experimental setup used in this study, showing **a** the control (CK), **b** the snail residue (SR), and **c** the lime (SL) treatments

significant soil compaction was observed in the SL treatments at levels of lime addition equal to or above 25 g kg^{-1}. Therefore, the results of SL treatments with addition levels above 2.5 g kg^{-1} are not presented in this study.

Soil sampling and analysis

At the end of the incubation, soil in each pot was sampled using a stainless soil auger (2.8 cm in internal diameter, 20.0 cm in length) with a hand shank. Sampled fresh soils were sealed in plastic packaging bags, transferred to the laboratory, and stored at 4 °C. Each soil sample was mixed thoroughly and screened through a 2-mm plastic mesh to remove plant roots and organic residue generated during the incubation period. Part of each fresh soil sample was used for extracellular enzyme assay. For further phospholipids fatty acids (PLFA) analysis, part of each soil sample was transferred into a −20 °C freezer and stored for 12 h and then freeze-dried with a lyophilizer. Another part of each soil sample was air-dried, ground, and passed through a 0.25-mm screen for analysis of soil pH, TOC, TN, NH_4-N, NO_3-N, and available phosphorus (AP).

Soil pH was measured (1:2.5, soil:water) with a handheld Multiparameter meter SX-620 (San Xin, China). A 20-mg air-dried and powdered soil sample was used for TOC and TN analysis with a Vario micro Cube elemental analyzer (Elementar, Germany). The concentrations of NH_4-N and NO_3-N were determined using an UV–Vis spectrophotometer following the methods described by Dong et al. (2018) with a wavelength of 420 nm and a double wavelength of 275 nm and 220 nm. The content of TP was measured using the method described by Andersen (1976). The content of AP in each soil sample was measured following the molybdenum-antimony anti-spectrophotometric method described by Bray and Kurtz (1945).

Extracellular enzyme assay

Four enzyme activities were assayed following the fluorometry method described by Bell et al. (2013). Specifically, the activities of β-1,4-glucosidase (BG), β-D-cellobiosidase (cellulose degradation; CB), β-1,4-*N*-acetylglucosaminidase (NAG), and acid phosphatase (ACP) related to C, N, and P cycling were determined. A 1.5-g fresh soil was dissolved with 125 mL of acetic acid buffer solution in a porcelain evaporating dish and blended for 30 min at 200 rpm with a magnetic stirrer (JB-3, Jintan Ronghua Instrument Manufacture Co., Ltd., Jiangsu province, China). Then, a blended 200 μL of soil buffer solution was injected into a deep well plate (Labtide, Greystone Biosciences LLC, USA). Meanwhile, 50 μL of acetic acid buffer solution was injected into a deep-well plate as the blank well plates, 50 μL of enzyme substrate was injected as the sample well plates, and 50 μL of 4-methylumbelliferyl (MUB) was injected as the quench well plates. Two hundred microliter of the acetic acid

buffer with 50 μL of the enzyme-substrate was set as the negative well and 200 μL buffer plus 50 μL of MUB as the reference standard well. After thorough mixing, all samples were incubated for 3 h at 37 °C in an incubator (RXZ, Dongqi, China). Before analysis, samples were centrifuged for 3 min at 2900 rpm (Eppendorf, USA) and transferred to a black flat-bottomed 96-well microplate. Fluorescence measurement was conducted in a microplate reader (SYNERGY H1, BioTek, USA) setting the excitation wavelength of 365 nm and an emission wavelength of 450 nm.

Determination of PLFA

PLFA analysis technique was applied to characterize the soil microbial community (Bossio and Scow, 1998). Potassium phosphate, chloroform, and methanol buffers were used to extract total lipids from the freeze-dried soil samples (8 g) that were sieved (< 2 mm) and completely mixed. A Silica Column (500 mg, Sigma, Germany) was used to fractionate phospholipids from neutral fatty acids and glycolipids. Fractionated samples were dissolved in hexane, and a 7890-gas chromatography outfitted with a Sherlock Microbial Identification System (V. 6.2, MIDI Inc., Newark, DE, USA) was used to identify the phospholipids. To determine and calculate the content (nmol g^{-1} dry soil) of individual PLFA, the fatty acid 19:00 was added to the fractionated samples before assay as the internal standard.

We used standard fatty acid nomenclature to name the identified PLFAs. For example, the designation 18:1w9c indicates that the compound has 18 carbons and one double bond, and "ω9c" notation refers to the 9th carbon from the "omega" or "ω" end of the chain. The sum of the total amount of the fatty acids was calculated to identify microbial species. The fatty acids containing anteiso- or iso- were summed as the amount of Gram-positive (G+) bacteria; mono-unsaturated, hydroxyl, and cyclopropane fatty acids were summed as Gram-negative (G−) bacteria; compounds with –COOH with $–CH_3$ on the tenth C were counted as actinomycetes (A); and 18:2ω6c and 18:1ω9c were calculated as fungi (F) (Bååth and Anderson, 2003; Wei et al., 2017). Bacteria (B) were calculated as the sum of G+ and G− bacteria. Finally, the sum of all PLFA biomarkers was calculated to represent total microbial biomass.

Analysis of variance (ANOVA) followed by a Duncan test was applied to compare the differences between the treatments. When assumptions of normality and homogeneity of variance were not met, data were rank-transformed using the Rankit method to obtain normal scores for data analysis. Spearman correlations were conducted based on the results of a Shapiro-Wilk normality test to evaluate the correlation between soil properties and microbial properties (Table 2). Redundancy analysis (RDA) was performed for testing correlations between soil physicochemical properties and microbial

Table 2 Spearman's rho correlations (*r*) between soil physicochemical properties, microbial properties, and extracellular enzymatic activities

	Biomass	G+	G−	A (%)	B (%)	F (%)	G+/G−	B/F	B/A	A/F	BG	CB	NAG	ACP
NO_3-N	0.333	0.368*	0.006	− 0.375*	0.471**	− 0.472**	0.316	0.503**	0.386*	0.290	0.023	0.164	0.228	0.003
AP	− 0.174	− 0.331	0.266	0.244	− 0.286	0.140	− 0.340	− 0.209	− 0.232	− 0.098	0.010	− 0.042	− 0.087	− 0.426*
TOC	0.566***	0.354	0.095	− 0.677***	0.443*	− 0.477**	0.322	0.499**	0.704***	0.079	0.145	0.410*	0.460*	0.160
TN	0.363*	0.465**	0.074	− 0.518**	0.512**	− 0.620***	0.426*	0.611***	0.535**	0.329	0.107	0.281	0.305	0.256
pH	0.535**	0.014	0.206	− 0.620***	0.163	− 0.167	0.011	0.190	0.622***	− 0.245	0.509**	0.624***	0.621***	− 0.111
NH_4-N	0.586***	0.254	− 0.058	− 0.650***	0.312	− 0.362*	0.267	0.366*	0.624***	0.004	0.147	0.424*	0.469**	0.351
NH_4-N/NO_3-N	− 0.258	− 0.386*	0.004	0.317	− 0.478**	0.490**	− 0.329	− 0.520**	− 0.333	− 0.361*	0.032	− 0.118	− 0.192	0.011
TP	0.546**	0.324	0.169	− 0.493**	0.460*	− 0.380*	0.240	0.415*	0.487**	0.152	− 0.095	0.124	0.189	0.160

NO_3-N: nitrate nitrogen; AP: available phosphorus; TOC: total organic carbon; TN: total nitrogen; NH_4-N: ammonium nitrogen; TP: total phosphorus; G+: Gram-positive bacteria; G−: Gram-negative bacteria; A: actinomycetes; B: bacteria (the sum of G+ and G−); F: fungi; BG: β-1,4-glucosidase; CB: β-D-cellobiosidase; NAG: β-1,4-*N*-acetylglucosaminidase; ACP: acid phosphatase. Asterisks indicate that correlations are significant at the 0.05 (*), 0.01 (**), and 0.001 (***) levels (2-tailed)

variables. We set soil physicochemical properties as explanatory variables, and microbial species and their ratios as response variables. The further selection was handled to test which parameters had significant effects on microbial species and community structure (Table 3). Variance partitioning was used to analyze the impact of each significant soil property. To investigate the percent of variance explained by each significant factor, each factor was used as a constraint variable, while other significant factors were used as covariates (Ren et al., 2018). All statistical analyses were conducted using SPSS (V.22.0, SPSS, Chicago) and Origin Pro 2018 (Origin Lab Crop, USA). RDA was performed using Canoco 5.0.

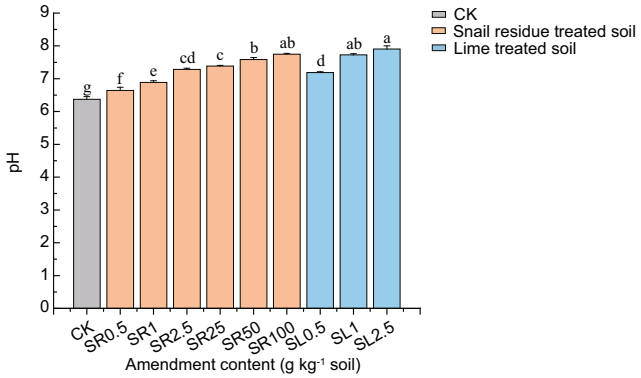

Fig. 2 Soil pH after the addition of snail residue (SR) and lime (SL) treatments. Significant differences among the treatments at the different addition levels are denoted by lowercase letters according to Duncan's multiple range test

Results and discussion

Effect of snail residue and lime on soil pH

Compared to the control (CK), both the snail residue (SR) and lime (SL) treatments significantly increased soil pH after the 120-day incubation (ANOVA, $F_{9,20} = 69.147$, $p < 0.001$; Fig. 2). More specifically, soil pH rose with increasing levels of SR and SL addition, though the rise in soil pH in the SL treatment was higher than in the SR treatment at the same addition level. For example, soil pH increased from 7.19 to 7.90 when lime was increased from 0.5 to 2.5 g kg^{-1}, but soil pH only increased from 6.64 to 7.28 for the same level of GAS residue addition. Further addition of GAS residue (25 to 100 g kg^{-1}) increased soil pH from 7.59 to 7.75.

The greater effect of the lime treatment than the snail residue treatment on soil pH was because lime produces more calcium hydroxide that neutralizes hydrogen ions. However, there is a risk when lime is overused, because the addition of lime, especially above 1.0 g kg^{-1}, tends to alkalize soils, resulting in the sorption of AP and soil compaction (Kumar et al., 2007).

Effect of snail residue and lime on soil nutrients

The experimental addition of SR had significant effects on soil TOC and soil nutrients, whereas SL did not (Table 1). The contents of TOC (ANOVA, $F_{6,14} = 15.83$, $p < 0.001$) and NO_3-N (ANOVA, $F_{6,14} = 152.39$, $p < 0.001$) both increased significantly with the addition of SR (Table 1). The highest content of NO_3-N was 38.29 mg kg^{-1}, found at the SR addition level of 25 g kg^{-1}. The contents of both TN (ANOVA, $F_{6,14} = 36.71$, $p < 0.001$) and NH_4-N (ANOVA, $F_{6,14} = 15.44$, $p < 0.001$) were significantly greater than the control at 25 g kg^{-1} and above in the SR treatment (Table 1). Significant variation was also observed in the contents of TP (ANOVA, $F_{6,14} = 6.51$, $p < 0.001$) and AP (ANOVA, $F_{6,14} = 5.91$, $p < 0.001$) among the different treatments.

The significant rise in NO_3-N and TOC contents in the SR treatment occurred because the snail residue contains snail meat with proteins (54%, specifically C: 23.02%, N: 0.58%, and P: 0.46%, determined in this study) (Bombeo-Tuburan et al., 1995), which released NO_3-N and TOC after decomposition (Keenan et al., 2018). Our results were similar to another study in which elevated contents of TOC, TN, NH_4-N, and P were found in animal by-product-amended soils (Cayuela et al., 2009). The lower increase in NH_4-N content found only in the very high SR addition could be caused by the loss of volatile NH_4-N during the processing and preparation of the snail residue (Mahimairaja et al., 1994; Erisman et al., 2008). Phosphorus sorption and desorption processes depend on many factors and soil conditions, such as soil pH, SOM content, P content, and soil type, as well as aluminum (Al) and iron (Fe) content (Pal, 2011; Yan et al., 2013; Debicka et al., 2016; Gérard, 2016). Here, TP content increased significantly with greater SR addition, possibly because of the high P content in the GAS residue. In contrast, the lowered AP content that occurred in SR while elevated AP content found in SL treatments was probably attributed to the significant changes observed in SOM. GAS residue contains abundant OM such

Table 3 Summaries of redundancy analysis of microbial species, microbial community structure, and soil physicochemical properties

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.6454	0.0141	0.0012	0.0004
Explained variation (cumulative)	64.54	65.96	66.08	66.12
Pseudo-canonical correlation	0.82	0.5723	0.6134	0.4963
Explained fitted variation (cumulative)	97.58	99.72	99.91	99.96

as proteins and amino acids (Mochida, 1991; Marsyha et al., 2018), which likely drove the significant decrease of AP observed at the addition of 100 g kg^{-1} SR, though both the lime and GAS residue treatments induced high soil pH (Table 1). For example, Debicka et al. (2016) reported that the removal of SOM decreased P sorption capacity, indicating that SOM plays an important role in P binding and limited P leaching from sandy soils. Although SOM was not measured in this study, TOC was analyzed and is a good predictor of SOM to some extent (Ostrowska and Porębska, 2012).

The snail residue and lime treatments both drove a V-shape response in the ratio of NH_4-N:NO_3-N (Table 1). Specifically, the ratio of NH_4-N:NO_3-N declined in SR from the CK to the addition of 25 g kg^{-1} and then rose with increasing SR addition from 25 g kg^{-1} to 100 g kg^{-1}. In the SL treatment, a similar decreasing and then increasing trend with SL addition was observed (Table 1). These particular responses likely occurred because soil pH impacts the oxidation process of NH_4-N to NO_3-N, and the gradual input of $CaCO_3$ with increasing amount of SR and SL treatments, as previously reported that the application of $CaCO_3$ decelerates the oxidation process (Dancer et al., 1973). For instance, in this study, a threshold value of 7.5–7.9 limited the oxidation of NH_4-N to NO_3-N. Soil NO_3-N and NH_4-N both increased with addition from CK to 50 g kg^{-1} SR, because more NH_4-N and/or NO_3-N were released by the snail residue. However, the increase in GAS residue also drove up soil pH levels so that they inhibited the oxidation of NH_4-N to NO_3-N. The significant increase in the ratio of NH_4-N:NO_3-N at 2.5 g kg^{-1} SL was mainly due to the decline in NO_3-N, although NH_4-N also increased slightly (Table 1). A similar V-shape observed in the SL treatment possibly resulted from the high pH and more $CaCO_3$ induced by the increasing addition of the lime.

TN content decreased at the addition of 25 g kg^{-1} SR and above. This decrease could be attributed to the decline in the oxidation of NH_4-N to NO_3-N, as discussed above, with NH_4-N increasingly volatilized or transformed to NO_2 and NH_3 and removed from the system, indicated by the rise in NH_4-N observed at higher GAS residue addition levels. NH_3 volatilization and N_2O emissions are the main pathways of N loss (Pan et al., 2016; Woodley et al., 2018).

It should be noted that considering water (with dissolved nutrients) leaking from the bottom of the pots, the ability of GAS to elevate nutrient levels could be under-estimated in our experiment (i.e., it is possible that GAS could raise levels of nutrients in natural rice fields even higher with limited leakage).

Effect of snail residue and lime on microbial biomass

The experimental addition of snail residue and lime had significant positive effects on microbial biomass (ANOVA, $F_{6,14}$ = 9.05, $p < 0.001$; Fig. 3). Amendment levels (0.5–50 g kg^{-1}) of

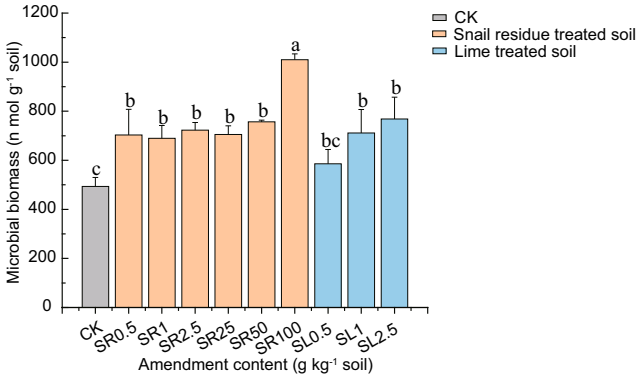

Fig. 3 Microbial biomass of the control (CK), snail residue treated soil (SR), and lime treated soil (SL). Significant differences among the treatments at different addition levels are indicated by different lowercase letters according to Duncan's multiple range test

SR and (0.5–2.5 g kg^{-1}) SL almost doubled microbial biomass to around 700 nmol g^{-1} dry soil, and the addition of 100 g kg^{-1} SR increased the biomass to 1,010 nmol g^{-1} dry soil.

Leff et al. (2015) reported that elevated N and P inputs led to increases in the relative abundance of faster growing copiotrophic bacterial taxa and decreases in the relative abundance of mycorrhizal fungi and methanogenic archaea. Given this, in our study, elevated TOC and nutrients such as TP, TN, NH_4-N, and NO_3-N content could improve the soil environment for some bacteria (copiotrophic bacterial taxa) by providing more substrates and nutrients. Similarly, our results showed that G+ and G− bacteria together accounted for more than 50% of the total microbial biomass. Previous studies have shown positive relationships between pH and biomass in a UK silty loam soil and that soil microbial biomass was greatest above pH 7 (Pietri and Brookes, 2008). In our study, microbial biomass also rose in response to increasing soil pH in both the SR and SL treatments (Fig. 3). Our results were consistent with those reported by Cayuela et al. (2009), whose results suggest that the addition of animal by-products produced an immediate and remarkable increase of mineral N (NH_4-N) in the soil, and this improvement induced an exponential increase in the soil respiration rate, reflecting the growth of microbial biomass.

The experiment showed significant effects on soil microbial species relative abundance, based on PLFAs (Fig. 4a). Compared to the control, G+ bacteria decreased, while fungi increased in both SR (ANOVA, $F_{6,14} = 7.53$, $p = 0.001$; Fig. 4a) and SL (ANOVA, $F_{3,8} = 5.64$, $p = 0.023$; Fig. 4a) treatments between 0.5 and 25 g kg^{-1} amendment levels of GAS residue and lime. At equivalent levels of SR and SL, the only difference was a slightly lower relative abundance of actinomycetes at 2.5 g kg^{-1} SL. However, higher levels of SR increased the relative abundance of G+ (27%) and total bacteria (58%) and decreased the relative abundance of fungi (< 10%) and actinomycetes (< 10%), compared to lower levels of SR. Similar to abundance, there was little effect of

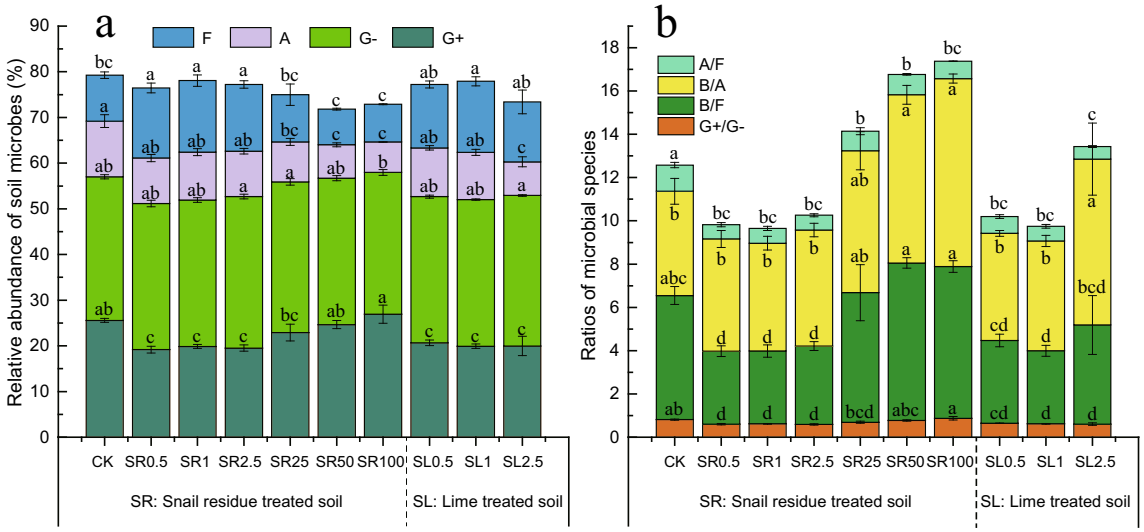

Fig. 4 The relative abundance of microbial species (**a**) and the ratios of microbial species (**b**) for different snail residue (SR) and lime (SL) treatments. Microbes are indicated as follows: sum of G+ and G− (B), Gram-positive bacteria PLFAs (G+), actinomycetal PLFAs (A), Gram-negative bacteria PLFAs (G−), and fungal PLFAs (F). B/A represents the ratio of bacteria to actinomyces, B/F represents the ratio of bacteria to fungi, A/F represents the ratio of actinomyces to fungi, and G+/G− represents Gram-positive to Gram-negative bacteria. Significant differences among the treatments at different addition levels are indicated by different lowercase letters according to Duncan's multiple range test

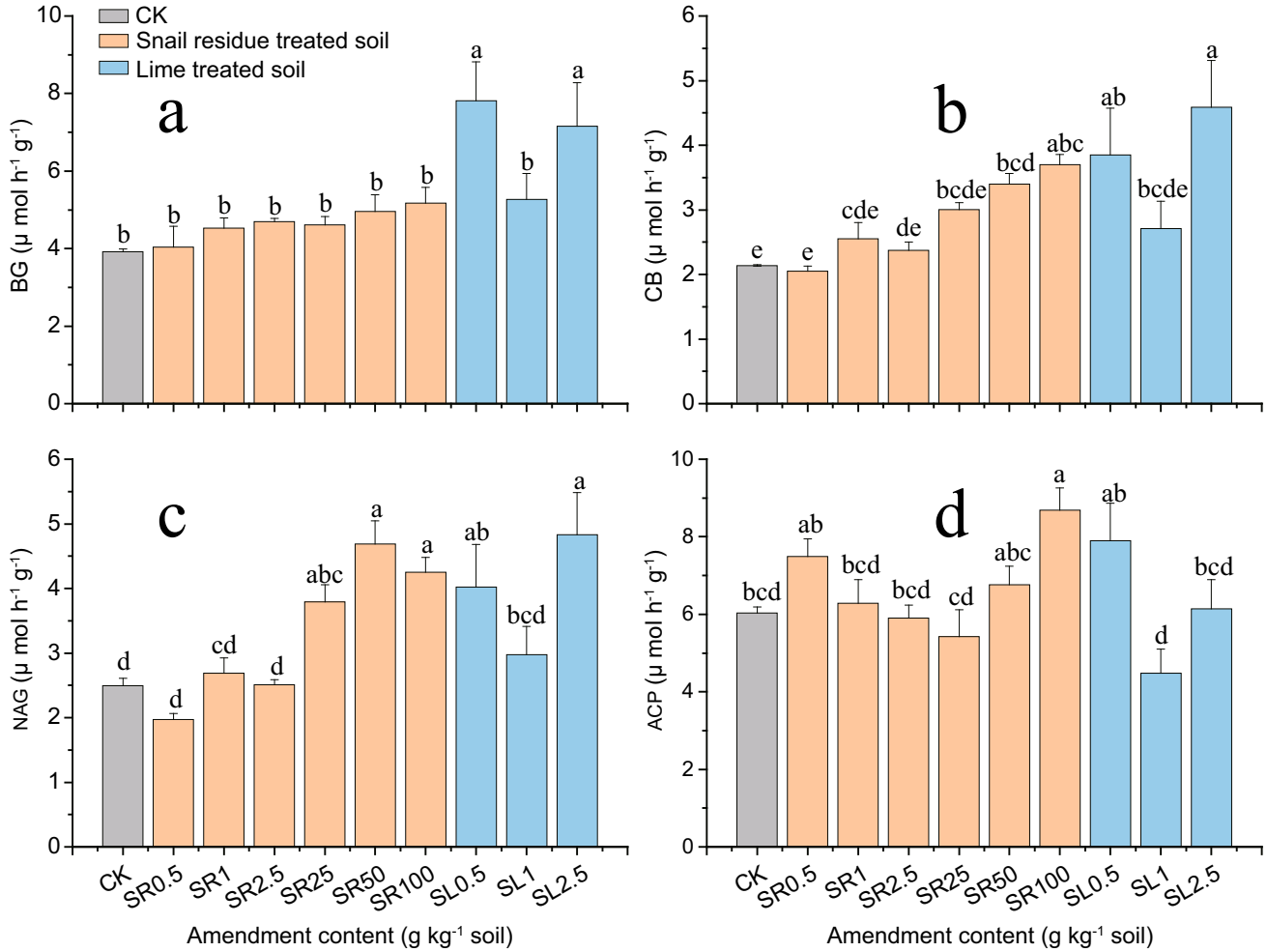

Fig. 5 Soil enzyme activities at different snail residue (SR) and lime (SL) treatment levels. ACP stands for acid phosphatase, BG for β-1,4-glucosidase, NAG for β-1,4-*N*-acetylglucosaminidase, and CB for β-D-cellobiosidase (cellulose degradation). Significant differences among the treatments at different addition levels are indicated by different lowercase letters according to Duncan's multiple range test

the addition of SR and SL on the ratios of microbial species (Fig. 4b). In both SR and SL, the ratios of B:F and A:F were lower than the control, driven by the changes in the relative abundances of G+ and fungi mentioned previously (Fig. 4a). Comparing equivalent levels of SR and SL, B:A was greater at 2.5 g kg^{-1} in SL than SR.

The application of snail residue had a consistent effect on the diversity of soil microbes. Specifically, the relative abundance of G+ and G− bacteria increased, while F and A substantially declined at increasing levels of with GAS residue addition, though the relative abundance of G+ was lower than CK at 0.5 to 2.5 g kg^{-1} (Fig. 4). Similar trends were observed in the SL treatment, though these were not significant (Fig. 4). Possible explanations for these differences could be the variation in soil nutrients, TOC, and pH at different SR levels (Rousk et al., 2010; Leff et al., 2015; Zhalnina et al., 2015; Xing et al., 2019). For example, Leff et al. (2015) reported that the input of N and P could decrease the relative abundance of mycorrhizal fungi, and methanogenic and oligotrophic bacterial taxa, but increase that of faster-growing, copiotrophic bacterial taxa. In our study, the addition of GAS residue also served as N and P fertilizer to provide soil microbes with more N and P, inducing higher relative abundance of G+ and lower relative abundance of fungi. Further, Rousk et al. (2010) found that both the relative abundance and diversity of bacteria were positively related to pH, while the relative abundance of fungi was unaffected by pH and fungal diversity was only weakly related with pH. Zhou et al. (2016) found that long-term nitrogen fertilization decreased fungal diversity and altered fungal community composition. Considering these effects, increasing soil pH can also contribute to raising soil bacterial (the sum of G+ and G−) relative abundance, while TOC and soil nutrients drive the decline in fungal relative abundance.

Effect of snail residue and lime on enzyme activity

SR and SL treatments induced considerable variations in CB (ANOVA, $F_{6,14} = 19.68$, $p < 0.001$; $F_{3,8} = 4.00$, $p = 0.052$; Fig. 5b), NAG (ANOVA, $F_{6,14} = 22.27$, $p < 0.001$; $F_{3,8} = 4.16$, $p = 0.048$; Fig. 5c), and ACP (ANOVA, $F_{6,14} = 4.93$, $p = 0.007$; $F_{3,8} = 4.08$, $p = 0.050$; Fig. 5d). Although BG increased at higher additions of GAS residue, no significant differences were observed, likely because of variation in soil pH and inputs of C, N, and P. Specifically, for BG, CB, and NAG, soil pH played the dominant role in regulating these extracellular enzyme activities, while the labile or non-labile nutrients (P) were responsible for the fluctuation of ACP. More specifically, at higher soil pH (under GAS residue addition of 25 g kg^{-1}), ACP was inhibited through more P input (Fig. 5). At higher GAS additions, however, ACP activity increased and eventually had even greater activity than the control (CK). For example, Acosta-Martinez and Tabatabai (2000) investigated the effects of lime application on the activity of 14 soil enzymes in field conditions and found that,

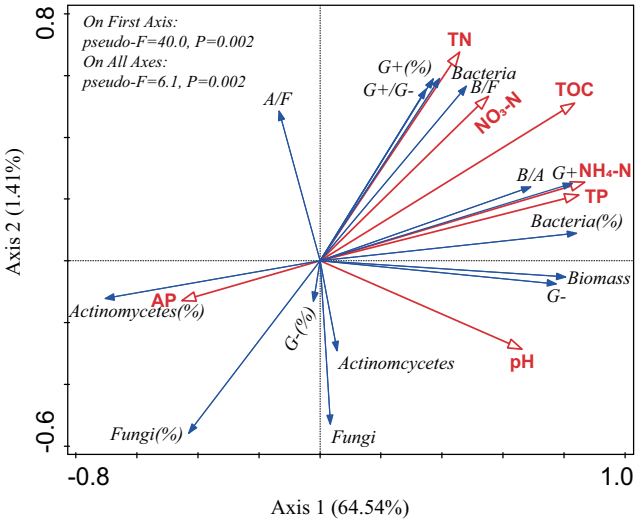

Fig. 6 Redundancy analysis of soil properties, including pH, contents of total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), nitrate nitrogen (NO_3-N), ammonium nitrogen (NH_4-N), and microbial properties involving microbial biomass, bacteria (B), fungi (F), actinomycetes (A), and the ratios of microbial species after golden apple snail residue amendment and incubation for 120 days

with the exception of ACP (which was significantly and negatively correlated with pH), the activities of all the other enzymes were significantly and positively affected. Here, our results also showed similar variation for all enzymes, including an increasing trend in ACP at GAS addition over 25 g kg^{-1}.

For the SL treatment, a V-shape change trend was observed for all enzymes (Fig. 5). All enzyme activity was higher at 0.5 g kg^{-1} lime addition than the CK because of the more appropriate soil pH environment. However, the lime addition caused a significant decline of all enzyme activity at 1 g kg^{-1}, possibly attributed to the decrease or change in form of C, N, and P (Chapin et al., 2003) (Fig. 6; Table 4). Further addition of lime to 2.5 g kg^{-1} led to the elevation of all enzyme activities, probably because of the low availability of assimilable nutrients. Economic theories of microbial metabolism (Tasoff

Table 4 Summary of variance partitioning analysis of soil properties (i.e., pH, TN, TP, AP, NO_3-N, NH_4-N) on the variation in microbial properties

Name*	Explains (%)	Contribution (%)	Pseudo-*F*	*P*
NH_4-N	48.70	73.60	26.50	0.002
pH	8.30	12.50	5.20	0.016
NO_3-N	4.30	6.50	3.10	0.080
TP	4.00	6.00	2.60	0.110
TOC	0.80	1.20	0.50	0.500
TN	0.10	0.20	< 0.10	0.854
AP	< 0.10	< 0.10	< 0.10	0.962

*Full names of abbreviations: ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), total phosphorus (TP), total organic carbon (TOC), total nitrogen (TN) and available phosphorus (AP)

et al., 2015) predict that enzyme production should increase when simple nutrients are scarce and complex nutrients are abundant; however, other soil properties such as compaction (Mariani et al., 2006; Miransari et al., 2009), drought (Hale et al., 2005), and salinity (Hu and Schmidhalter, 2005) would constrain the availability of labile nutrients. In our study, lime addition induced soil compaction at levels over 1.0 g kg^{-1}, as previously reported (Kumar et al., 2007), and therefore, more enzymes were generated and higher enzyme activities were induced in order to target sufficient nutrients.

Cost-effectiveness and limitations

Even though the addition of GAS residue exhibited promising effects on the bio-control of GAS and benefits to soil remediation, we would like to highlight some specific areas for further investigation. For example, soils for our pot experiments were sampled from paddy fields which had a different environment, and the pH of the soil used in our study already fell in the range of the optimal pH values, so the effects of GAS residue on strongly acidic soil, such as found in paddy fields, are still unknown, which is needed to further study. In the meantime, we also need to do a series of pretreatments before adding GAS residue to soils, including picking GAS from paddy fields, freezing to kill GAS, drying and smashing, mixing with soil, and watering for incubation; these processes are not cost-effective and practicable for most farmers. However, these step-by-step treatments demonstrated the possibility of using GAS residue as fertilizer and soil conditioner. Although the field application of GAS residue has not been verified here, our study provides a new pioneer model for the biocontrol of GAS. To make this biocontrol method more practical and cost-effective, a suggested field method is to collect GAS from paddy field and then transfer them to dryland before plowing, leave them exposed to sunlight on the surface of dryland for a few days, and then plow with a high-speed rotary tiller to thoroughly grind and mix the GAS residue and soil. After that, let the GAS residue undergo natural decomposition process for a few days before planting crops. This proposed approach should be further explored for practical application in the future.

Conclusions

We conclude that the application of powdered GAS residue to input and mix non-irrigated farmland soil releases nutrients, and alleviates soil acidity almost as well as lime; moreover, it provides more resources to soil microbes to increase their bioactivity. The recommended optimum levels of GAS residue addition were 1–25 g kg^{-1} soil, not only for soil properties, but also for soil microbes. Overuse of GAS residue resulted in TN loss and AP sorption by raising soil pH and TOC content. Regression analysis and RDA suggested that elevated soil properties are responsible for variations in soil microbial species, community composition, and bioactivity. Specifically, NH_4-N and soil pH accounted for more than 85% of the variation in microbial properties. Our results suggest that GAS residue can be used as a fertilizer and soil amendment, which may contribute to the control of the invasive snail in many parts of the world, as well as the remediation of barren and acidic soils. Farmers and other users should determine the balance of labor and cost-effectiveness prior to using the biocontrol method developed in this study.

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