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Soil biological quality in rhizosphere, growth, and yield of upland rice grown on acid soil after amended biochar enriched sap of *Kappaphycus alvarezii*

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Abstract. *Rakian TC, Kilowasid LMH, Afa LO, Riskyana A, Nurazizah, Wijayanti Y, Bahrun A, Subair I, Rahni NM, Alam S, Sarawa, Karimuna L. 2023. Soil biological quality in rhizosphere, growth, and yield of upland rice grown on acid soil after amended biochar enriched sap of* Kappaphycus alvarezii. *Biodiversitas 24: 6780-6792.* Biological soil quality is essential in evaluating soil management practices using biochar enriched for upland rice growth on acid soil. Therefore, the study aimed to (i) analyze the effect of biochar-enriched sap of *Kappaphycus alvarezii* (K-sap) on the abundance of Arbuscular Mycorrhizae Fungi (AMF), bacteria, and protozoa in rhizosphere soil, growth, and yield of upland rice, and (ii) the relationship between the abundance of soil biota and the growth and yield components, as well as straw N, P, and K content on acid soil. Four upland rice were grown on acid soil incubated with biochar-enriched sap for two weeks in pots. Nine treatments of the biochar-enriched sap were tested, namely control, 5% biochar-0% K-sap, 5% biochar-5% K-sap, 5% biochar -10% K-sap, 5% biochar-15% K-sap, 7.5% biochar-0% K-sap, 7.5% biochar-5% K-sap, 7.5% biochar-10% K-sap, and 7.5% biochar-15% K-sap. Each treatment was three replicates in a randomized block design. The results demonstrated that the AMF spores quantity decreased while flagellates increased in the soil rhizosphere. The P and K content increased, the N:P decreased, and P:K ratio varied in the straw of the upland rice. Plant height and total spikelets increased, while root dry weight and roots to shoots ratio (R:S) decreased with biochar enriched. The abundance of AMF spores and flagellates correlated positively and negatively with R:S. Furthermore, R:S was negatively correlated with plant height and total spikelets. Plant height and total spikelets showed a positive correlation, while root dry weight and R:S was negatively correlated with K content in shoot tissue. The P and K contents with N:P ratio and N:P with P:K were negatively correlated. In conclusion, biochar-enriched K-sap effectively regulated the activity and composition of soil biota in the rhizosphere, influencing upland rice's growth, yield, and nutrient balance uptake on acid soils.

Keywords: Control, nutrient balance, ratio, seaweed extract, soil biota, uptake

INTRODUCTION

Soil biological quality is an important indicator in sustainable agricultural management. Soil biota, including bacteria, Arbuscular Mycorrhizal Fungi (AMF), and soil fauna, are essential in managing carbon stocks and fertility for sustainable food crop productivity (Bonilla-Bedoya et al. 2023). Rice is the primary carbohydrate source in Southeast Asia (Bandumula 2018) and can be produced by small farmers in Indonesia (Sujarwo and Hanani 2016). Upland rice contributes to the economic resilience of the families of thousands of small farmers (Murniati and Mutolib 2020), and it is cultivated using traditional cultivars' seeds. Southeast Sulawesi province has a variety of cultivars grown on acid soil (Kikuta et al. 2020; Kilowasid et al. 2021). In this area, the managing soil fertility practice still applies crop cultivation systems through slash-and-burn, cropping, and fallow cycles (Kikuta et al. 2016). Therefore, optimal production only takes place in the short term, and restoring the soil quality through the fallow system consumes a lot of time (Ying et al. 2018; Temjen et al. 2022).

High acidity and low organic carbon content contribute significantly to poor soil fertility (Barus 2016). Nutrient availability is a service contributed by the ecological activity of bacteria, fungi, mycorrhizae, protozoa, and nematodes in the soil ecosystem (Bennett and Klironomos 2018). The organic matter addition can increase the pH and carbon content, which can have a long-term positive impact on acid soil quality services to upland rice (Kikuta et al. 2018; Gafur and Umran 2019; Krauss et al. 2020). Biochar use is receiving attention as an organic matter source to amend agricultural soil quality (Ayaz et al. 2021; Das et al. 2023). Adding biochar can improve soil water-holding capacity, moisture, structure, and pH to support biota activities in nutrient cycling on acid soil (Hossain et al. 2020). The enhancement of biochar's agronomic performance within soil quality improvement practices frequently entails its combination with various soil amendments. These amendments may include inorganic fertilizers, organic liquid fertilizers, microbial inoculants, or compost (Cox et al. 2021; Peng et al. 2021; Tian et al. 2021). The mixed or enriched biochar is richer in its nutrient content than the

biochar matrix (Hagemann et al. 2017). Applying the biochar enriched with liquid organic fertilizer increases microbial metabolic activity, water and nutrient retention in soil, and plant growth (Syahrinudin et al. 2019; Holatko et al. 2021).

Using liquid organic fertilizer in biochar enrichment strategies to improve plant nutrient uptake, fertilizer use efficiency, and soil biota activity control continue to be developed (Ndoung et al. 2021). Recently, seaweed extract has been used as a liquid organic fertilizer applied through the leaves or into the soil (Vafa et al. 2022). The seaweed *Kappaphycus alvarezii* liquid extract (K-sap) contains plant growth-promoting substances and essential nutrients for plant growth (Basavaraja et al. 2018; Deolu-Ajayi et al. 2022). The seaweed *K. alvarezii* is cultivated by small farmers and is widespread in the coastal waters of Southeast Sulawesi (Kasim 2016; Aslan et al. 2020). The liquid extract of this seaweed (K-sap) can be used as a source of liquid organic fertilizer to enrich biochar to improve the soil biological quality in the rhizosphere and upland rice growth on acidic soils, especially in this region. Kilowasid et al. (2023a) reported that rice straw biochar enriched K-sap added to the soil increased pH, organic C, total N, available P, K, Ca, Mg, and exchangeable Na, Si, as well as flagellate density in the soil after incubated two weeks. However, further investigation is required to explore the impact of the modified chemical and biological characteristics on the rhizosphere soil biota and the growth and yield of upland rice. This study aimed to analyze (i) the impact of acid soil amended with K-sap-enriched biochar on the abundance of Arbuscular Mycorrhizal Fungi (AMF), bacteria, and protozoa in rhizosphere soil, as well as on the growth and yield of crop, and nutrient content, and (ii) the relationship between the abundance of the soil biota, growth, yield, and nutrient content in upland rice on the acid soil amended.

MATERIALS AND METHODS

Soil collection and analysis

Soil samples was taken from up to 10 cm deep, collected from the Ultisols order in the local upland rice cultivation area in Wolasi District, Konawe Selatan Regency, Southeast Sulawesi (Kilowasid et al. 2023a). The soil physicochemical properties are listed in Table 1.

Experimental site, treatments, and design

The experiment was conducted in a greenhouse at the Faculty of Agriculture, Halu Oleo University, from March to October 2021. The treatment of biochar-enriched K-sap was as follows: (i) control (B0-S0), 5% biochar with 0% K-sap (B5-S0), 5% biochar with 5% K-sap (B5-S5), 5% biochar with 10% K-sap (B5-S10), 5% biochar with 15% K-sap (B5-S15), 7.5% biochar with 0% K-sap (B7.5-S0), 7.5% biochar with 5% K-sap (B7.5-S5), 7.5% biochar with 10% K-sap (B7.5-S10), and 7.5% biochar with 15% K-sap (B7.5-S15). Furthermore, each treatment was repeated three times and randomly arranged according to the randomized block design procedure.

Enriching biochar with K-sap and incorporating it into the soil

Biochar was produced from rice straw, which was pyrolyzed at 370°C for 4 hours in a cylindrical reactor. The granules passed through the sieve size <150 µm per opening, and then adsorbed with K-sap was extracted from fresh *K. alvarezii* aged 35 days (Kilowasid et al. 2023a). This extract was stored at 4-5°C as a stock solution (100% concentration) until use. Nutrient content in the stock solution included Zn, Fe, Mn, Mg, Ca, Cu, Na, K, P, and S at 0.06 ppm, 76.90 ppm, 0.10%, 0.67%, 0.06%, 0.10 ppm, 0.85%, 0.68%, 0.02%, and 0.40% determined using ICP-OES (Kilowasid et al. 2023a). In addition, the nitrogen determined by the Kjeldahl method was 0.23%.

The stock solution was diluted using water into four concentrations (v/v): 0%, 5%, 10%, and 15%. Every K-sap solution was mixed with biochar at a ratio of 500 mL K-sap/kg biochar (Kilowasid et al. 2023a) and the physicochemical characters were presented in Table 2.

Every enriched biochar was mixed with 10 kg of soil passing a sieve of <4 mm/opening inserted into a different pot. The pot was placed randomly in a glass house according to the experimental design. The soil in the pot was watered until saturated and incubated for two weeks, and the moisture content was maintained at 35% during the incubation period.

Table 1. The physicochemical character of soil samples.

Soil parameters	Unit	Method	Value
Particle fraction:			
Sand	%	Pippete	11*
Silt	%	Pippete	61*
Clay	%	Pippete	28*
$pH_{(H2O)}$	-	1:5	4.2*
$pH_{(KCl)}$	-	1:5	3.8
Electrical conduct.	dS/m	1:5	0.18*
Organic C	%	Walkley and Black	1.28*
Total N	%	Kjeldahl	0.11*
CN-ratio	-		12*
Total P_2O_5	mg/100 g	25% HCl	32*
Available P_2O_5	ppm	Bray 1	12.6
Total K_2O	mg/100 g	25% HCl	7.0*
Exchangeable Ca	cmol/kg	1 N NH_4-Acetate, pH 7	0.66
Exchangeable Mg	cmol/kg	1 N NH_4-Acetate, pH 7	0.25
Exchangeable K	cmol/kg	1 N NH_4-Acetate, pH 7	0.12
Exchangeable Na	cmol/kg	1 N NH_4-Acetate, pH 7	0.27
CEC	cmol/kg	1 N NH_4-Acetate, pH 7	8.56*
Base Saturation	%	1 N NH_4-Acetate, pH 7	15.0*
Al^{3+}	cmol/kg	1 N NH_4-Acetate, pH 7	3.47*
H^+	cmol/kg	1 N NH_4-Acetate, pH 7	0.53*
Si	ppm	Acetate buffer	36.0*

Note: *From Kilowasid et al. 2023a. CEC is cation exchangeable capacity

Table 2. Physico-chemical characteristics of K-sap enriched-biochar

Parameter	Unit	Method	Value from enriched biochar with different concentrations of K-sap			
			S0	**S5**	**S10**	**S15**
Organic C	%	Ash/gravimetry	24.92	24.50	24.12	22.48
Total nitrogen	%	Kjehdahl/destilation	2.56	2.23	2.23	2.50
C/N	-		10	11	11	9
Others material	%	Gravimetry/sieve mesh	0.00	0.00	0.00	0.00
Water content	%	Gravimetry/oven	39.5	38.1	38.4	36.5
pH_{H2O}	-	Potentiometry/pH meter	9.4	9.3	9.3	9.6
Available P_2O_5	%	Citrate 2%/Spectrophotometry	0.81	0.87	0.91	0.91
Available K_2O	%	Citrate 2%/F-AAS	2.0	1.8	1.7	2.1
CEC	$cmol_c\ kg^{-1}$	$NH_4OAc\ pH_7$-Spectrophotometry	35.30	33.02	30.28	35.07
Humic compound	%	Spectrophotometry	0.22	0.21	0.28	0.33
SiO_2	%	Na_2CO_3 dan NH_4NO_3/F-AAS	0.39	0.40	0.38	0.38

Note: CEC is cation exchangeable capacity

Planting and maintenance

After two weeks of incubation, the soil was planted with seeds of Wakawondu, a local upland rice cultivar. Four seeds primed in water for twelve hours were planted 5 cm deep from the soil surface with a distance of 10 cm between plants. In line with the seeds planted, 2.0 g of urea, 1.5 g of SP-36, and 1.5 g of KCl were applied to each pot between the rows of plants. Irrigation was conducted when the soil moisture content was <35% and weeds were removed by hand, while pests were controlled using insecticides.

Plant observation and sampling of soil and roots

At 162 days after planting, plant height and panicle length were measured, and productive tillers, total grain per panicle, and percentage of filled grain were counted. The soil in each pot was carefully separated from the roots, and 500 g of soil was used for AMF spore analysis. Soil attached to the root surface was taken using tweezers to determine the abundance of bacteria and protozoa. The soil mixed with the roots was put into a container filled with tap water, stirred gently, and then the suspension was poured over a double-arranged filter.

A total of 30 pieces of fresh roots were selected randomly and placed in different Petri dishes to observe root infection. The roots and shoots rinsed with distilled water were dried at room temperature and at 60°C for 48 hours in an oven. The total dry weight of the plant was obtained from the sum of the dry weight of roots and shoots. Meanwhile, the root-to-shoot ratio (R:S) was determined from the ratio between root and shoot dry weights.

Abundance determination and morphology identification of soil bacterial colonies

For each treatment, 1 g of soil from the root surface was added to 9 mL of distilled water in a test tube, vortexed, and then serially diluted 10-2 to 10-6 (Pang et al. 2020). 50 µL of the diluted suspension was dropped on the Nutrient Agar (NA) plate, spread, and incubated for 48 hours under room temperature. The total growing bacterial colonies were counted manually and expressed as colony-formed units (CFU)/g soil (Vieira and Nahas 2005; Lee et al. 2021). Different colony isolates were sub-cultured on other NA mediums for 24 hours under room conditions. Colony morphological characters were identified based on pigment color, shape, margins, size, and elevation (Leboffe and Pierce 2011; Borkar 2018). One entire loop of each isolate was dripped with 10 µL of 3% KOH on a glass slide and stirred. Meanwhile, when a gel was formed within 5-60 seconds, it was categorized as gram-negative; otherwise, it was gram-positive (Powers 1995; Sudewi et al. 2020).

Determination of AMF spores abundance and root infection

50 g of soil was carefully combined with 500 mL of water and manually stirred until a homogeneous mixture was achieved. The suspension was poured over a multilevel filter from top to bottom at 212, 63, and 38 µm per opening. Furthermore, water from the faucet was carefully poured over all parts of the top filter surface until it came out through the bottom filter. The soil retained in the 63 and 38 µm filters was transferred to a centrifuge tube, added with 20% and 60% sugar solutions, which was vortexed and spun at 2000 rpm for 3 minutes. Subsequently, the liquid in the tube was transferred to a 38 µm filter and poured with water slowly until all the sugar solution was washed out. Spores were transferred to a Petri dish to be counted at 30 randomly selected fields of view under a stereomicroscope (Kilowasid et al. 2023b). AMF spores with different colors and shapes were transferred to glass slides and mounted with Meltzer's reagent, and the morphology was identified following the guidelines of Brundrett et al. (1996).

The roots were also washed using water and cut into 2 cm lengths. The pieces were sterilized in 60% ethanol, left for 5 minutes in a test tube, and then rinsed using distilled water. In addition, the roots were transferred to a test tube containing 10% KOH, heated at 90°C for 10 minutes, rinsed with water, and dried under room conditions. They were soaked in 2% HCl for 20 minutes and rinsed with distilled water (Sarkodee-addo et al. 2020). Subsequently, the roots were marked with 0.05% aniline blue and heated at 85°C for 4 minutes. The colored roots were rinsed using

water and then stored in glycerol. A total of 30 pieces of root were transferred to a glass slide and covered with a cover glass to be observed under a microscope. The percentage of root infection was estimated based on the presence of vesicles and internal and external hyphae that appear blue in the root tissue (Dalpé and Séguin 2013).

Flagellates abundance counting

A total of 1 g of soil was transferred to a Petri dish (5 cm in diameter) containing 5 mL of distilled water and allowed to stand until the soil particles were dispersed for 1-2 minutes under room conditions. Furthermore, 15 µL of the soil suspension was transferred to a hemocytometer, covered, and scanned under a microscope at 400 times magnification. Flagellates that appeared in the appropriate grid were counted and the abundance was expressed in the number of cells per gram of soil (Syaf et al. 2021).

Measurement of N, P, and K contents in shoots

After the dry weight was measured, the shoots were ground to determine the N content using the Kjeldahl method; P was extracted with HNO_3 and measured using a spectrophotometer, while K was obtained with HNO_3 and measured using flame-atomic absorption spectrophotometry.

Statistical analysis

The data were subjected to one-way ANOVA at the level of $p<0.05$. When significant at $p<0.05$ level, the Duncan Multiple Range Test (DMRT) was applied to detect differences between treatments. Pearson's bivariate correlation was used to determine the nature of the relationship between components of soil biota, growth, yield, and N, P, and K content in shoots.

RESULTS AND DISCUSSION

Soil biota in rhizosphere soil

Results of ANOVA showed that the acidic soil incubated for two weeks with K-sap enriched biochar treatment had a significant (at $p<0.05$ level) effect on number of AMF spores and flagellate cells, while the abundance of total bacteria and root infection was non-significant ($p>0.05$) in the soil of the rhizosphere Wakawondu upland rice.

Three bacterial isolates were found in soil treated with K-sap enriched biochar, namely *Pseudomonas* sp.1, *Pseudomonas* sp.2, and *Serratia* sp. (Figure 1).

In Table 4, the morphological characters of the bacterial colony isolate *Pseudomonas* sp.1 (Figure 1A) growing on NA medium show yellow pigment. The colony size is moderate, the general shape is circular, the margin is intact, and the elevation is convex. *Pseudomonas* sp.2 (Figure 1B) shows a moderate-sized white pigment; the general shape is circular and margin, while the elevation is convex. *Serratia* sp. (Figure 1C) shows red pigment, punctiform size, irregular general shape, undulate margin, and convex elevation. The three bacterial isolates were categorized into the group of gram-negative bacteria.

The presence of each isolate in the rhizosphere soil of the Wakawondu cultivar upland rice from each treatment of K-sap enriched biochar is presented in Table 5. In Table 5, the B5-S10 treatment allowed the presence of the three bacterial isolates in the rhizosphere of the Wakawondu local cultivar upland rice with an incubation period of 2 weeks. *Pseudomonas* sp.1 and sp.2 appeared in the soil from the rhizosphere treated with B0-S0 and B7.5-S5. *Pseudomonas* sp.1 and *Serratia* sp. were found in the rhizosphere of soils treated with B5-S15. Meanwhile, *Pseudomonas* sp.2 and *Serratia* sp were found in the B5-S5 treatment. In the soil rhizosphere of upland rice cultivars grown on acid soils treated with B5-S0 and B7.5-S0, which were incubated for 2 weeks, only *Pseudomonas* sp.2 was found.

A **B** **C**

Figure 1. Colony morphology of isolates. A. *Pseudomonas* sp.1, B. *Pseudomonas* sp.2, C. *Serratia* sp.

Table 3. The abundance of bacteria, protozoa, AMF spores in soil from the rhizosphere, and root infection of local cultivar Wakawondu upland rice on acid soil after incubation for two weeks with K-sap enriched biochar treatment

Symbols of treatment	Parameters of soil biological quality in rhizosphere (mean±sd, n = 3)			
	Total Bacteria ($x10^6$ CFU g^{-1} soil)	AMF spore ($x10^2$ spore 50 g^{-1} soil)	Root infection (%)	Flagellate ($x10^9$ cell g^{-1} soil)
B0-S0	62.00±42.57a	49.24±10.31d	61.11±5.85a	2.06±1.66a
B5-S0	46.67±15.28a	30.52±4.49a	58.89±14.17a	8.98±2.91b
B5-S5	48.00±16.37a	36.30±6.83c	67.22±4.81a	10.56±0.35b
B5-S10	32.67±20.23a	32.83±4.32bc	66.11±18.28a	11.00±1.37b
B5-S15	36.00±27.78a	33.81±4.90bc	70.56±14.37a	8.60±1.04b
B7.5-S0	30.67±16.65a	23.76±3.00abc	61.67±10.14a	7.31±3.85b
B7.5-S5	51.33±12.06a	23.86±7.08ab	71.11±13.37a	10.48±4.76b
B7.5-S10	38.00±27.78a	25.32±3.12ab	72.78±16.78a	7.63±3.91b
B7.5-S15	29.33±11.72a	21.50±1.94a	52.00±10.48a	7.89±2.67b

Note: Numbers in the same column followed by different letters show significant differences according to DMRT at the $p<0.05$ level

Table 4. Morphological characters of bacterial colonies isolated from rhizosphere soil of Wakawondu upland rice grown on acid soil, which had been incubated for two weeks with K-sap enriched biochar treatment

Morphological character of bacteria colony	Soil bacteria isolate		
	***Pseudomonas* sp.1**	***Pseudomonas* sp.2**	***Serratia* sp.**
Color pigment	Yellow	White	Red
Size	Moderate	Moderate	Punctiform
General form	Circular	Circular	Irregular
Margins	Intact	Intact	Undulate
Elevation	Convex	Convex	Convex
Grams	Negative	Negative	Negative

Table 5. The presence of each bacterial isolate in the soil from the rhizosphere of the local Wakawondu upland rice cultivar grown on acid soil incubated for two weeks with K-sap enriched biochar treatment

Treatment	Soil bacteria isolate		
	***Pseudomonas* sp.1**	***Pseudomonas* sp.2**	***Serratia* sp.**
B0-S0	1	1	0
B5-S0	0	1	0
B5-S5	0	1	1
B5-S10	1	1	1
B5-S15	1	0	1
B7.5-S0	0	1	0
B7.5-S5	1	1	0
B7.5-S10	0	1	0
B7.5-S15	0	1	0

Note: 0 is absent, 1 is present

Data in Table 3 show that the total abundance of AMF spores in B0-S0 was the densest, while B7.5-S15 was the least abundant. The order of AMF spore density levels from densest to rarest according to treatment is as follows: B0-S0>B5-S5>B5-S15>B5-S10>B5-S0>B75.5-S10>B7.5-S5=B7.5-S0>B7.5-S15. Meanwhile, the difference in AMF spore density between B0-S0 and B7.5-S15 and others is significant. The number of AMF spores in the B5-S5 treatment compared to B5-S0, B7.5-S5, B7.5-S10, and B7.5-S15 was significantly different, while compared to B5-S10, B5-S15, B7.5-S0 was insignificant. The AMF spore density B5-S15 differed substantially from that of B7.5-S5, B7.5-S10, and B7.5-S15 was significant, while compared to B5-S10 and B7.5-S0 was non-significant. The density of AMF spores in B5-S10 did not differ significantly from that of B7.5-S5 and B7.5-S10. The AMF spore densities of B7.5-S10, B7.5-S5, and B7.5-S0 B7.5-S15 differed substantially. A total of six genera of arbuscular mycorrhizal fungi spores (Figure 2) were found in all rhizosphere soils of the Wakawondu local cultivar upland rice grown on acid soil, which had been incubated for two weeks with K-sap enriched biochar. Furthermore, each genus was found in all treatments of K-sap-enriched biochar.

Table 3 also shows the percentage of local upland rice roots infected by AMF from the highest to the lowest rate of root infections in the following sequence: B7.5-S10>B7.5-S5>B5-S15>B5-S5>B5-S10>B7.5-S0>B0S0>B7.5-S15. The difference in the root infection percentage between the K-sap enriched biochar treatments was insignificant.

The abundance of flagellates from the most to the least sequential is B5-S10>B5-S5>B7.5-S5>B5-S0>B5-S15>B7.5-S15>B7.5-S10>B7.5-S0>B0-S0. The flagellates abundance of B5-S10 was significantly different from that of B0-S0 but was insignificant compared to the others. The abundance between other treatments compared to B0-S0 was also significantly different, as shown in Table 3.

Plant growth component

Acidic soil incubated for two weeks with K-sap enriched biochar treatment had a significant effect (ANOVA at $p<0.05$ level) on rice plant height, root dry weight, and the ratio of roots to shoots, while the shoot dry weight and total plant dry weight were non-significant. Table 6 shows that the tallest plants (110.69 cm in height) occurred in treatment B7.5-S10, while the shortest plants (83.26 cm in height) were shown in B0-S0. The order from tallest to shortest decreased according to the treatment as follows: B7.5-S5>B7.5-S10>B5-S10>B5-S15>B7.5-S0>B7.5-S15>B5-S5>B5-S0>B0-S0. The plant height at B7.5-S5 was significantly different from that at B0 but insignificant compared to others. Plant height at B5-S10 differed significantly from B0-S0 but was non-significant with others. Plant heights of B0-S0, B5-S0, B5-S5, B5-S15, B7.5-S0, and B7.5-S15 were non-significant.

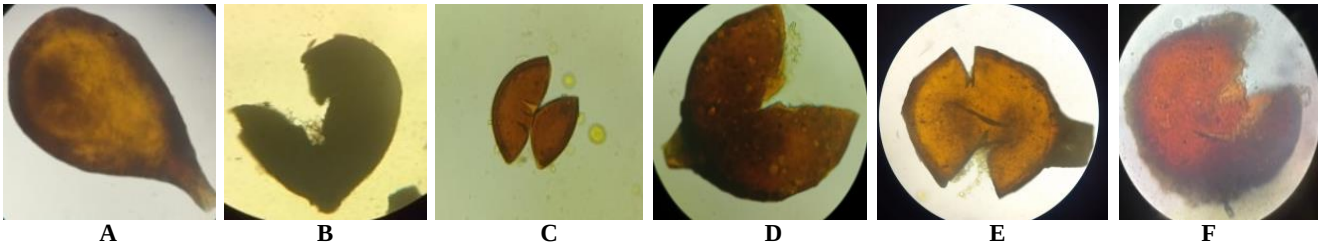
The order of root dry weight from the heaviest to the lightest decreased according to the treatment as follows B0-S0=B7.5-S15>B5-S0>B5-S10>B5-S5>B7.5-S0>B7.5-S5>B7.5-S10>B5-S15 (Table 6). The difference in root dry weight in the B0-S0 and B7.5-S15 treatments compared to the B7.5-S10, B7.5-S5, B7.5-S0, B5-S5, and B5-S15 treatments was significant but was insignificant with others. The root dry weight of B5-S0 differed significantly from that of B7.5-S10 and B5-S15 but did not significantly differ from that of B5-S10, B5-S5, B7.5-S0, and B7.5-S5. The difference in root dry weight between treatments B5-S10, B5-S5, B7.5-S0, B7.5-S5, B7.5-S10, and B5-S15 was insignificant.

Table 6 shows the ratio of roots to shoots dry weights at B0-S0, which is the heaviest, while B7.5-S10 is the lightest. The order of root-to-shoot dry weight ratio, from highest to smallest decreased according to the treatment as follows: B0-S0 (0.57)>B7.5-S15 (0.30)>B5-S0 (0.26)>B5-S5 (0.27)>B5-S10 (0.24)>B7.5-S0 (0.22)>B5-S15 (0.21)>B7.5-S5 (0.18)>B7.5-S10 (0.15). Percentage reduction of R:S for treatments B7.5-S15, B5-S0, B5-S5, B5-S10, B7.5-S0, B5-S15, 7.5-S5, and B7.5-S10 compared to B0-S0 (control) are 48, 49, 53, 58, 61, 63, 68, and 74%, respectively. The difference in R:S at B0-S0 compared to B7.5-S10 and other treatments is significant. The value of R:S at B7.5-S15 compared to B7.5-S10 was significantly different but was non-significant with others, except for B0-S0. The R:S differences between B5-S5, B5-S10, B5-S15, B7.5-S0 and B7.5-S5 were not significant.

Table 6. Growth components of upland rice grown on acid soil, which was incubated for two weeks with K-sap enriched biochar treatments

Symbols of treatment	Plant height (cm)	Root dry weight (g)	Shoot dry weight (g)	Plant (root+shoot) dry weight (g)	Root to shoot ratio
B0-S0	83.26±15.79a	38.17±7.51c	66.83±14.63a	105.00±20.75a	0.57±0.09c
B5-S0	89.64±6.75abc	32.33±11.41bc	121.00±37.99a	153.33±36.26a	0.29±0.14b
B5-S5	92.81±5.66abc	24.67±5.80ab	93.17±19.37a	117.83±23.38a	0.27±0.06ab
B5-S10	106.08±6.66bc	27.50±3.50abc	117.00±36.59a	144.50±39.47a	0.24±0.06ab
B5-S15	100.41±18.84abc	18.17±5.86a	92.33±16.65a	110.50±13.87a	0.21±0.09ab
B7.5-S0	100.04±8.06abc	23.50±9.54ab	105.17±25.85a	128.67±34.82a	0.22±0.05ab
B7.5-S5	111.94±9.07c	21.50±2.29ab	120.33±11.86a	141.83±13.80a	0.18±0.01ab
B7.5-S10	110.69±2.71c	19.17±3.33a	133.67±43.02a	152.83±45.87a	0.15±0.03a
B7.5-S15	94.56±9.14abc	38.17±1.61c	127.00±10.82a	165.17±11.54a	0.30±0.02b

Note: Numbers in the same column followed by different letters show significant differences according to DMRT at the $p<0.05$ level



A B C D E F

Figure 2. Spore genera of AMF found in the rhizosphere soil of the local Wakawondu upland rice cultivar: A. *Glomus* sp.1, B. *Glomus* sp.2, C. *Glomus* sp.3, D. *Acauluspora* sp., E. *Gigaspora* sp.1, F. *Gigaspora* sp.2

Reproductive component associated with yield

ANOVA results showed that treatment with K-sap enriched biochar significantly affected the total spikelets and the percentage of filled grain per panicle. In contrast, the panicle length was insignificantly affected. The highest number of spikelets per panicle occurred in treatments B5-S15. Conversely, the least occurred in B0-S0. The order of the total spikelets per panicle from the highest to the lowest number decreased according to the treatment as follows: B5-S15>B7.5-S5>B7.5-S15>B5-S10>B5-S5>B7.5-S0>B5-S0>B7.5-S10>B0-S0. The highest number of spikelets in B5-S15 occurred in B0-S0, and the difference between the two treatments was significant. Meanwhile, significant differences in total spikelets also occurred between B5-S15 compared to B7.5-S10 but were not significant compared to other treatments. The total number of spikelets of B7.5-S10 differed significantly from that of the B0-S0 treatment, but it was insignificantly different from that of other treatments (Table 7).

Table 7 shows a significant difference in the spikelet filled/panicle percentage between treatments. The order of rate of spikelet filled from the most decreased to the least was as follows: B0-S0> B7.5-S15> B5-S0> B7.5-S5> B5-S15> B5-S5> B5-S10> B7.5-S10> B7.5-S0. The difference in the percentage of filled spikelets of B0-S0, B7.5-S0, B7.5-S10, and B5-S10 was significant but was not significant compared to B7.5-S15, B5-S0, B7.5-S5, B5-S15, and B5-S5. The lowest percentage of filled spikelets in treatment B7.5-S0 differed significantly from that of B5-S0, B7.5-S5, B5-S15, B5-S5, B5-S10, and B7.5-S10.

Nutrient content in the shoot

ANOVA showed that the K-sap enriched biochar treatment significantly affected the P, K, N:P, and P:K in upland rice shoots. In contrast, the N content and N:K ratio were insignificantly affected. In Table 8, the order of P content in upland rice shoots from the most to the least decreases according to the treatment as follows: B5-S5>B7.5-S0= B7.5-S15> B5-S0= B5-S15> B5-S10= B7.5-S10> B7.5-S5> B0-S0. The difference in P content of upland rice tissue of B5-S5, B7.5-S10, B5-S10, B7.5-S5, and B0-S0 was significant but not significant compared to B7.5-S0, B7.5-S0, B5-S15, and B5-S0. The tissue P content of B7.5-S0 and B7.5-S15 differed significantly with that of B7.5-S5 and B0-S0 but was not significant compared to B5-S0, B5-S15, B5-S10, and B7.5-S10. The difference in P content between treatments B5-S10, B7.5-S10, B7.5-S5, and B0-S0 was insignificant.

The order of K content in upland rice shoots from the most decreased to the least according to the treatment was as follows: B7.5-S10>B7.5-S5>B5-S5>B7.5-S0>B5-S10>B5-S15>B7.5-S15>B5-S0>B0-S0. The K content of upland rice tissue at B7.5-S10 differed significantly from that of B0-S0 and B5-S0 but was non-significant with others. The K content at B5-S0 was significantly different from B0-S0 but was non-significant compared to others, except for B7.5-S5 and B7.5-S10 treatments.

The N:P ratio in shoots sequentially from highest to lowest was as follows: B0-S0>B7.5-S5>B5-S10>B5-S15>B7.5-S10> B7.5-S15> B7.5-S0> B5-S0> B5-S5. The difference in the ratio of N:P in shoots at B0-S0 compared to the other treatments is significant. The N:P ratio at B7.5-

S5 differed significantly from that of B5-S5 and B5-S0 but was insignificant with others, except B0-S0. The difference in the N: P ratio at B5-S10 and B5-S5 was significant but non-significant with the others, except compared to the B0-S0 treatment. The lowest N:P ratio occurred at B5-S5 but was not significantly different from that of B5-S0, B5-S15, B7.5-S0, B7.5-S10, and B7.5-S15.

The P:K ratio in shoots from highest decreased to lowest according to the treatment as follows: B5-S5=B5-S0=B7.5-S15>B5-S15=B7.5-S0>B0-S0>B5-S10=B7.5-S10>B7.5-S5. The P:K ratio of upland rice tissue in B5-S5 differed significantly from B0-S0, B5-S10, B7.5-S10, and B7.5-S5 but was non-significant compared to others. The difference in the P:K ratio of upland rice tissue of B5-S15 and B7.5-S5 differed significantly with B7.5-S0 but was non-significant compared to B0-S0 and B7.5-S10 treatments.

Relationship between nutrient content in shoot, AMF, growth, and yield

Pearson's correlation analysis (Table 9) showed that there was a significant positive relationship (r = 0.59*) between plant height and total grains per panicle and K content in shoots of upland rice (r = 0.48*). Meanwhile, there was a significantly negative correlation between root dry weight (r = -0.44*) and R:S (r = -0.51*) with plant height. Root dry weight correlated significantly positively with R:S (r = 0.74*), and negatively correlated with K content in shoots (r = -0.49*). There was a positive significant correlation between R:S and AMF spore abundance in rhizosphere soil (r = 0.69*) and the N: P ratio in shoots, but significantly negative with total spikelets per panicle (r = 0.54*), flagellate density (r = 0.46*), and K shoot content (r = -0.73*). The total spikelets per panicle correlated positively with flagellate density (r = 0.51*), and K shoot content (r = 0.45*), but was negatively correlated with AMF spore density (r = -0.47*). The percentage of spikelet filled did not show a real positive or negative correlation with other parameters. AMF spore density was positively correlated with the N:P ratio in shoots (r = 0.45*), while it was significantly negatively correlated with the K content of plant tissue (r = -0.56*). There was a significant positive correlation (r = 0.65*) between flagellate density and the content of K shoots, and a significant negative correlation (r = -0.52*) with the N:P ratio of shoots. A negative correlation existed between P (r = - 0.74*) and K (r = - 0.53*) content with N:P in shoots. The relationship between the N:P and P:K ratios in shoots was negatively correlated (r = -0.53*).

Table 7. Reproductive components are associated with upland rice yield on acid soil, which has been incubated for two weeks with K-sap enriched biochar treatments

Symbols of treatment	Productive tillers number per-clump	Panicle length (cm)	Total spikelet (grain) per-panicle	Percentage of spikelet filled per-panicle
B0-S0	7.83±0.76a	20.43±1.10a	59.13±4.57a	31.41±5.24bc
B5-S0	9.08±1.12a	21.25±0.42a	80.70±3.66bc	23.60±6.49abc
B5-S5	9.42±1.18a	21.88±1.37a	84.45±12.60bc	18.40±6.82ab
B5-S10	9.42±1.44a	22.21±0.74a	86.10±8.57bc	18.27±2.71a
B5-S15	8.17±0.52a	22.36±1.90a	97.54±10.96c	19.71±5.75ab
B7.5-S0	8.67±0.88a	20.97±2.53a	81.89±7.63bc	14.76±2.76a
B7.5-S5	9.58±1.18a	22.28±0.72a	94.07±11.00bc	22.02±4.49abc
B7.5-S10	9.58±1.61a	20.40±1.59a	77.08±9.32b	16.94±1.47a
B7.5-S15	9.17±2.45a	22.04±0.91a	88.40±11.24bc	28.50±6.37bc

Note: Numbers in the same column followed by different letters show significant differences according to DMRT at the p<0.05 level

Table 8. Content of nitrogen (N), Phosphorous (P), Potassium (K), and the N: P, N: K, and P: K ratio in upland rice straw on acid soil which has been incubated for two weeks with K-sap enriched biochar treatments

Symbols of treatment	N (%)	P (%)	K (%)	N:P	N:K	P:K
B0-S0	0.56±0.19a	0.06±0.02a	1.59±0.23a	10.11±2.19d	0.35±0.11a	0.04±0.02abc
B5-S0	0.67±0.29a	0.18±0.04bcd	3.36±0.68b	3.54±1.05ab	0.21±0.11a	0.06±0.02cd
B5-S5	0.79±0.22a	0.26±0.02d	4.19±0.11bc	3.04±0.67a	0.19±0.06a	0.06±0.01d
B5-S10	0.72±0.19a	0.12±0.03abc	3.76±0.37bc	6.52±2.69bc	0.20±0.06a	0.03±0.01abc
B5-S15	0.85±0.07a	0.18±0.04bcd	3.74±0.26bc	4.90±0.59abc	0.23±0.03a	0.05±0.01bcd
B7.5-S0	0.87±0.21a	0.20±0.04cd	4.17±0.20bc	4.59±1.94abc	0.21±0.05a	0.05±0.01bcd
B7.5-S5	0.70±0.15a	0.10±0.01ab	4.47±0.99c	7.01±1.02c	0.17±0.08a	0.02±0.01a
B7.5-S10	0.50±0.09a	0.12±0.07abc	4.53±0.39c	4.73±2.05abc	0.11±0.02a	0.03±0.02ab
B7.5-S15	0.92±0.43a	0.20±0.09cd	3.61±0.41bc	4.65±0.24abc	0.26±0.13a	0.06±0.02cd

Note: Numbers in the same column followed by different letters show significant differences according to DMRT at the p<0.05 level

Table 9. Pearson correlation between nutrient content in shoots, AMF, flagellate, growth, and yield components

	PH	RDW	R:S	TS	PSF	AMF	Flag	P	K	N:P
PH										
RDW	**-0.44***									
R:S	**-0.51***	**0.74***								
TS	**0.59***	-0.34	**-0.54***							
PSF	-0.31	0.33	0.21	0.02						
AMF	-0.35	0.34	**0.69***	**-0.47***	0.03					
Flag	0.27	-0.19	**-0.46***	**0.51***	-0.18	-0.19				
P	-0.07	-0.18	-0.30	0.34	-0.02	-0.33	0.20			
K	**0.48***	**-0.49***	**-0.73***	**0.45***	-0.33	**-0.56***	**0.65***	0.28		
N:P	0.08	0.24	**0.50***	-0.32	0.18	**0.45***	**-0.52***	**-0.74***	**-0.53***	
P: K	-0.36	0.05	0.01	0.14	0.21	-0.10	-0.10	**0.86***	-0.20	**-0.53***

Note: PH: Plant Height, RDW: Root Dry Weight, R:S: Root to Shoot ratio dry weight, TS: Total Spikelets/panicle, PSF: Percentage of Spikelet Filled, P: Phosphorous, K: Potassium, N:P: Ratio Nitrogen to Phosphorous, P:K: Ratio Of Phosphorous to Potassium, AMF: Number of Arbuscular Mycorrhizal Fungi, and Flag: Number of flagellate cell

Discussion

The physicochemical characteristics of K-sap enriched biochar at concentrations of 0%, 5%, 10%, and 15% were similar. The results showed that acid soil incubated for two weeks with different doses had a significant effect on the total AMF spores and the abundance of flagellates in the rhizosphere of the local Wakawondu upland rice cultivar, while the abundance of bacteria and root infection by AMF was insignificant. The environmental conditions of the rhizosphere soil changed the total abundance of AMF and flagellate spores. Depending on the soil conditions, this phenomenon may either positively or negatively impact the presence of indigenous AMF spores (Panahi et al. 2020; Videgain-Marco et al. 2021). The positive effect of biochar-amended soil on total AMF spores often occurs in soils experiencing abiotic stress (Jabborova et al. 2021), while the negative impact on the form of a decrease in total AMF spores often occurs due to improvements in soil pH, P availability, and water availability (Liu et al. 2019; Xu et al. 2020). In this study, the total abundance of AMF spores in the rhizosphere of the local cultivar Wakawoundu upland rice cultivated on soil amended with K-sap enriched biochar was lower than without biochar (Table 1). Kalamulla et al. (2022) reported that adding biochar significantly reduced the total abundance of AMF spores in the rhizosphere of rice cultivated on unsterilized soil. In this study, the lower AMF spore abundance was related to an increase in pH, organic-C, total-N, C:N ratio, available-P, Ca, Mg, and exchangeable K, and Si content in acid soils after two weeks of incubation of biochar enriched and not enriched with K-sap (Kilowasid et al. 2023a). Table 1 showed that the reduction of the abundance of AMF spores increased with the amount of biochar added to the soil, both enriched and not enriched with K-sap. The most profound decrease in the total abundance of AMF spores occurred at a dose of 7.5% biochar by weight of the soil and enhanced with a K-sap concentration of 15%. The trend of decreasing total AMF spores in the rhizosphere soil of this upland rice cultivar with additional doses of rice straw biochar has been shown by Kilowasid et al. (2023b). This reduction in spore abundance was related to pH, organic-C, total-N, C:N ratio, available-P, Ca, Mg, and exchangeable K and Si content, which increased significantly with biochar doses (Posada et al. 2016; Hajiboland et al. 2018; Lermi and Palta 2022). The presence of K-sap at a concentration of 5% -15% in biochar at a dose of 5% supported the reduction in the abundance of AMF spores due to the addition of 5% biochar by weight of acid soil. The difference in total AMF spore abundance between K-sap concentrations for enriching biochar is possibly caused by the influence of different concentration levels of bioactive compounds on AMF spore sporulation in the soil (Al-Arjani et al. 2020). Therefore, enrichment of biochar with K-sap suppressed the negative effect of biochar up to a specific dose on the total abundance of AMF spores in the rhizosphere soil of this upland rice cultivar on acid soils. The presence of K-sap in biochar stimulated the germination of spores of arbuscular mycorrhizal fungi in acid soils by adding K-sap-enriched biochar. The study conducted by Kuwada et al. (2006) found that the extracts from red (including *Gricilaria verrucosa*, *Gelidium amansii*, and *Eucheuma cottonii*) and green algae (*Chlorella pyrenoidosa*) extracted in 25% methyl alcohol solution increased hyphal growth from AMF *Gigaspora margarita* and *Glomus caledonium* spores in vitro culture. Hines et al. (2021) reported the stimulation of spore germination of the AMF *Rhizophagus irregularis* by extracts of seaweed *Ascophyllum nodosum*, which contained bioactive compounds (such as alginate-derived oligosaccharides, phlorotannins, and mannitol) in the extract. Campos-López et al. (2022) also found that combinations of flavonoids and strigolactone with or without roots stimulated spore germination and the elongation and branching of hyphae of AMF. *K alvarezii* seaweed extract contains compounds with bioactivities, such as kinetin, auxin, and abscisic acid, which play a role in stimulating AMF sporulation and colonization, both symbiotic and asymbiotic (Liu et al. 2019; Vaghela et al. 2022). Therefore, the K-sap stimulated indigenous AMF sporulation in the rhizosphere soil of local upland rice due to the effect of reducing the abundance of AMF spores by increasing the dose of biochar on acid soils.

In the soil from the rhizosphere of upland rice, *Acaulospora*, *Gigaspora*, and *Glomus* spores were found

for all treatments, as shown in Figure 2. The three AMF genera were often dominant in the soil of the rhizosphere of upland rice and other plants (Olubode et al. 2020; Alimi et al. 2021; Baki et al. 2021). Sporulation and germination of AMF spores were colonized and then infected upland rice roots, as indicated by the presence of external hyphae, internal hyphae, vesicles, arbuscles, and spores (Campo et al. 2020). The difference in the percentage of root infection by AMF between treatments was insignificant (Table 3). Based on the total AMF spores abundance, the soil without biochar was higher than those with K-sap enriched biochar, but the percentage of infected roots between treatments was similar (Table 3). Therefore, adding K-sap to biochar stimulated the infective capacity of indigenous AMF spores in the rhizosphere of upland rice on acid soils. Marizal et al. (2016) found a positive relationship between the number of AMF spores and the degree of root infection of peanuts, but this phenomenon did not occur.

Meanwhile, Sefrila et al. (2021) reported the degree of AMF colonization negatively correlated with the phosphorus content of acid soils. After two weeks of incubation, the soil with K-sap enriched biochar had higher available P than without biochar (Kilowasid et al. 2023a). The phenomenon related to root infection by AMF was associated with the role of biostimulant substances carried by K-sap in biochar, which collaborated with phytohormone compounds released by roots to increase the ability of AMF spores in the soil to germinate, and hyphae to penetrate the roots of upland rice (Liu et al. 2019; Sugiura et al. 2020). Another possibility was related to the *Pseudomonas* sp. and *Serratia* sp. bacteria, which produced bioactivated substances with an auxiliary effect in increasing the infectivity of AMF hyphae on upland rice roots (Ordoñez et al. 2016; Nasslahsen et al. 2022).

The total abundance of bacteria was not significantly different between treatments (Table 3). Numerous studies have indicated that the disparity in the total abundance of soil bacteria between the control (without biochar) and experimental group (with biochar) was not statistically significant (Pressler et al. 2017; Zheng et al. 2019; Azeem et al. 2020). This phenomenon is related to the addition of biochar increasing the content of the recalcitrant organic carbon fraction in the soil, which is difficult for soil bacteria to access carbon and energy (de Vries and Caruso 2016; Wang et al. 2021a). The addition of biochar has a more significant effect on the composition of soil bacteria (Zhang and Shen 2022), which is caused by changes in soil pH, availability of organic carbon, and soil nutrients (Yin et al. 2021). In this study, *Serratia* sp. was exclusively detected in the rhizosphere soil treated with a 5% biochar dose enriched with K-sap at concentrations of 5%, 10%, or 15%.

Conversely, regardless of the biochar and K-sap concentrations, *Pseudomonas* sp. was observed in all treatment groups (Table 5). Therefore, biochar provides a suitable microhabitat for developing *Serratia* sp. and *Pseudomonas* sp. (Blanco-Vargas et al. 2022). K-sap also provides labile carbon stimulating *Serratia* sp. population growth at a dose of 5% biochar (Chen et al. 2022; Egamberdieva et al. 2022). Therefore, the presence of *Serratia* sp. and *Pseudomonas* sp. in the rhizosphere soil stimulates the germination of spores and the development of AMF hyphae to infect the roots of the Wakawondu local upland rice cultivar.

Root infection of upland rice by AMF indigenous helps the roots to take up more nutrients from the soil solution, affecting the content in the tissues and the growth of rice plants under aerobic and anaerobic conditions (Iqbal et al. 2020). The percentage of root infection was comparable among the different treatments. However, there was an observable trend: the highest phosphorus (P) content in shoots was recorded at a dose of 5% biochar enriched with a K-sap concentration of 5% (B5-S5). The highest potassium (K) content was observed with a biochar dose of 7.5% enriched with a K-sap concentration of 10% (B7.5-S10), and the lowest content was found in the treatment without biochar (Table 8). The results indicated that P was more available and taken up by upland rice roots when 5% biochar was applied and enriched with a 5% concentration of K-sap, particularly in acidic soils. For K, the treatment involving 7.5% biochar enriched with a 10% concentration of K-sap showed enhanced availability and uptake by upland rice roots. These findings suggested the potential benefits of biochar and K-sap amendments for nutrient uptake in acid soil conditions. Variations in P and K nutrient uptake can change the stoichiometry of N:P:K in the tissue of Wakawondu upland rice cultivars grown on acid soils amended with rice straw biochar (Kilowasid et al. 2023b). The N:P and P:K ratios varied between the K-sap enriched biochar treatments, as shown in Table 8. The N: P ratio in soil without the addition of K-sap-enriched biochar and 5% biochar enriched with K-sap concentration of 5% were the highest and lowest, respectively (Table 8). Therefore, adding K-sap enriched biochar increased the amount of P accumulated in the upland rice shoots on acid soils. The shoots N:P ratio correlated negatively ($r = -0.74^*$) with the shoots P content of this upland rice (Table 9). The amount of P compared to N accumulated in the upland rice shoots increased with the addition of K-sap-enriched biochar. A significantly positive correlation ($r = 0.50^*$) between the R:S and N:P ratio (Table 9) indicates that the amendment of acid soil with K-sap enriched biochar increased the amount of P compared to N translocated in the shoots of this upland rice.

The biomass accumulation in the roots more than in shoots explains the response of plants to a soil environment experiencing water and nutrient stress (Agathokleous et al. 2019). R:S values in the no-soil were higher than those with K-sap-enriched biochar (Table 6). The values positively correlated with AMF spore abundance and N:P ratio in shoots (Table 9). These phenomena show that photosynthate translocated to the roots more than the shoots in acid soil conditions. The photosynthate product was released into the rhizosphere through root exudate and rhizodeposit, which stimulated hyphal growth and the formation of new spores from AMF (Chowdhury et al. 2022). In Table 8, the N content of the fertilizer N was relatively similar between the treatments. This result might be related to the mineral nitrogen content between the K-sap enriched biochar treatments in the soil used for planting

upland rice (Kilowasid et al. 2023a). Sainju et al. (2017) found that the accumulation of biomass in the roots of perennial grasses was not affected by the amount of N added to the soil. Therefore, the positive correlation between R:S and N:P losses was controlled by the amount of P taken up by upland rice plants on acid soils. The amount of P in the fertilizer with the addition of K-sap enriched biochar was twice as high as without the addition of K-sap enriched biochar, except for the biochar dose of 7.5/kg soil enriched with K-sap concentration of 5%, which was only 1.7 times higher (Table 8). Therefore, N:P fertilizer ratio decreased with the addition of K-sap enriched biochar. This phenomenon was also demonstrated through a significant negative correlation (r = -0.74**) between the P content and N:P in shoots (Table 9). Table 9 shows the ratio N:P in shoots negatively related (r = -0.53**) with the ratio P:K in shoots. The phenomenon of the negative relationship between the N:P and P:K ratios were caused by high concentrations of K in the soil solution, inhibiting P uptake by plant roots (Wang et al. 2021b).

The abundance of flagellates in the rhizosphere soil between biochar, both enriched and without K-sap enrichment, was significantly higher than without biochar (Table 3). Liu et al. (2020) also found that the abundance of flagellates in soil from *Brassica napus* grown on acid soils amended with biochar was more abundant than without biochar from wheat straw. Furthermore, the increase in flagellate abundance was due to the indirect effect of increasing soil pH by adding biochar. Soil pH values affected the diversity and composition of soil bacteria (Chao et al. 2016; Shi et al. 2021), where bacteria were suitable prey for flagellates (Saleem et al. 2016). In this study, the increase in the abundance of flagellates was probably caused by a higher soil pH without biochar (pH was 4.77) (pH was 6.10-7.20) and the addition of biochar (Kilowasid et al. 2023a), affecting the abundance and composition of soil bacteria. Flagellate abundance was negatively correlated (r = -0.52*) with the ratio of N:P shoots, as shown in Table 9. Therefore, the increase in the flagellate population had implications for increasing the uptake of P more than N by the shoots, and the ratio of N:P shoots correlated significantly (r = 0.50*) with the R:S ratio. This fact indicated that an increase in R:S ratio triggered the ratio of N:P in shoots of upland rice. A higher R:S ratio indicated that more photosynthate was allocated to the roots to supply energy for the growth and development of upland rice roots under conditions of acid soil stress (Ouyang et al. 2021). Therefore, organic carbon compounds released by roots into the soil were increased to be used by bacteria and AMF. There was a significantly positive relationship (r = 0.69*) between R:S and total AMF spores but a significantly negative relationship (r = -0.46*) with flagellate abundance (Table 9). The phenomenon of a positive relationship between R:S and total AMF spores in soil from the rhizosphere of local upland rice cultivar Wakawondu grown on acid soils had been amended in rice straw biochar previously reported by Kilowasid et al. (2023b).

The amount of translocated nutrients in plant tissue dramatically determines the level of growth and yield components of upland rice plants. The K content in shoots was positively correlated with plant height (r = 0.48*) and total panicles per clump (r = 0.45*) while negatively correlated with root dry weight and R:S ratio (Table 9). This positive correlation reaffirmed that increasing the amount of K in the shoots increases plant height and the yield component of upland rice under acid soil conditions (Fageria and Oliveira 2014; Kilowasid et al. 2023b). The negative correlation between K shoots and root dry weight and R:S ratio indicates that increased K uptake in the shoots helps upland rice plants to buffer excessive allocation of biomass to roots in the face of stress conditions (Sustr et al. 2019). A high R:S ratio follows an increase in the amount of biomass allocated to the root, and this is indicated by a significantly positive correlation (r = 0.74*) between root dry weight and R:S ratio (Table 9).

In conclusion, the amendment of acid soil using K-sap-enriched biochar changed the character of biological quality in the rhizosphere region of Wakawondu's local upland rice cultivar. This was conducted by increasing the abundance of flagellates and reducing the total abundance of AMF spores. The changes in the abundance of AMF and flagellate spores were associated with a reduction in the roots to shoots dry weight ratio, which further altered the N:P and P:K balances in the shoots of the upland rice. This reduction in root-to-shoot ratio was followed by an increase in plant height and total spikelets. In addition, the interrelationship mechanism between the decrease in the percentage of filled grain and the balance of N, P, K in shoots, variations in the type of bacteria, and AMF should be studied for future study.

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