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Synthetic nitrogen coupled with seaweed extract and microbial inoculants improves rice (*Oryza sativa* L.) production under a dual cropping system

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Highlights

- *This study assessed the combined effect of seaweed extraction and microbial inoculants with reduced chemical fertilizer rates on the rice production.*
- *Seaweed extraction coupled with chemical fertilizer significantly improved biomass accumulation and grain yield of rice.*
- *Seaweed extraction and microbial inoculants, combined with a 20% reduction in nitrogen fertilizer, improved rice growth and yield.*
- *The correlation analysis revealed that the growth and yields traits significantly contributed to the higher grain yield.*
- *This study provides a sustainable nutrient management plan that increases crop production while minimizing costs of chemical N fertilizer application.*

Abstract

The over-reliance on synthetic nitrogen (N) in current farming is a major concern because of its adverse effects on soil quality, the environment, and crop production. Organic fertilizers such as seaweed extract (SE) and microbial inoculants (MI) provide alternatives to chemical fertilizers that could decrease the amount of synthetic N needing to be applied and improve crop growth productivity. This study evaluated the combined effect of SE and MI with reduced N rates on the growth, biomass accumulation, yield, and yield components of an N-efficient rice cultivar (Baixiang 139-A) and N-inefficient rice cultivar (Guiyu 9-B). Field experiments were conducted in the early and late growing seasons at different sites in Guangxi province, China, in 2019. A total of five treatments, such as T_1: N 180 + SE 0 + MI 0 (kg ha^{-1}) (control); T_2: N 180 + SE 3 + MI 3 (kg ha^{-1}); T_3: N 144 + SE 3 + MI 3 (kg ha^{-1}); T_4: N 126 + SE 3 + MI 3 (kg ha^{-1}); and T_5: N 108 + SE 3 + MI 3 (kg ha^{-1}) were used. The leaf area index (LAI), effective panicle number, grain per spike, grain filling rate, and 1000-grain weight were significantly increased in T_2 and T_3 compared with the control. The treatments T_2 and T_3 enhanced the biomass accumulation and grain yield of rice compared with the control. Furthermore, differences in the growth, yield, and yield components among the different cultivars were significant; however, there were no significant differences among the different locations. T_3 increased the LAI, grain filling rate, biomass accumulation, and grain yield of rice by 4.5%, 5.9%, 6.6%, and 5.2%, respectively, compared with the control. Improvements in grain yield were mainly attributed to the enhanced growth and yield components. The correlation analysis also confirmed that LAI, productive tillers, grain filling rate, and biomass accumulation were positively correlated with grain yield. In sum, T_3 [N144 + SE 3 + MI 3 (kg ha^{-1})] could achieve higher grain yield despite a reduction in the usage of chemical N. Generally, this study provides a sustainable nutrient management plan that increases crop production while minimizing costs of chemical N fertilizer application.

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Introduction

Rice (*Oryza sativa* L.) provides staple food for more than 60% of China's population (Yuan, 2014). Approximately 30.18 million hectares of rice were cultivated in China in 2018, accounting for 26% of the grain cultivated area nationally, and a total of 21.12

million tons of rice were produced (NBSC, 2018) China needs to produce 23,108 tons of rice by 2030 to meet the expected increase in per capita demand (Normile, 2008). Nitrogen (N) is important for plant growth, as N regulates crop yield by supporting the photosynthetic and sink properties of plants (Tubiello *et al.,* 2015; Wang *et al.,* 2016). The current farming system relies heavily on chemical N fertilizers to achieve higher yields. However, crop yield does not improve linearly with N fertilizer input (McGuire, 2015; Pathak *et al*., 2011), and extreme N fertilization poses major environmental concerns, such as increased greenhouse gas emissions, groundwater pollution, and soil quality deterioration (Mahajan *et al.,* 2008; Cai *et al.,* 2018). Furthermore, the long-term use of nitrogenous fertilizers has been shown to increase the acidification, deterioration, and compaction of arable soils, thus limiting plant growth and production (Yue *et al.,* 2012; Guo *et al.,* 2017). This continued dependency on chemical N fertilizer for agricultural crop production is not sustainable. There is thus, a need to implement sustainable management strategies that can feed the rising population and reduce environmental costs. The amount of N fertilization in China was estimated to be approximately 29.619 million tons, accounting for 21% of agricultural N applications globally (FAO, FAOSTAT, 2014). The average annual N fertilization rate is 180 kg/hm^2, which is 75% greater than the worldwide average annual N application rate for rice (Ma *et al.,* 2008; Peng *et al.,* 2009; Chen *et al.,* 2014) and exceeds the N fertilizer input for high-yield paddy fields (Peng *et al.,* 2006). China is a major consumer of N fertilizer, but the N utilization rate is not high (30-50%) (Zhao and Sha, 2014). Moreover, more than 45% of N fertilizers are lost to the ecological environment (Ju and Zhang, 2003; Ligeng and Weixing, 2002).

There is a growing interest in improving N-use efficiency to reduce costs without compromising crop productivity. Currently, the most challenging issue is to enhance grain yield, to feed the population on a sustainable basis with the least cost to the environment(Mueller *et al.,* 2012; Morone *et al.,* 2019). Several N fertilizer management techniques have been used, including optimal N dosage (Chen *et al.,* 2015), side-deep placement (Yao *et al.,* 2018), and slow-release N fertilization (Yang *et al.,* 2012). However, the implementation of these approaches has limitations because they are labour-intensive and lack of advanced technology (Anadon *et al.,* 2016). In contrast to traditional synthetic N fertilization, organic fertilizers are considered effective and environmentally friendly alternative fertilizers to improve crop productivity and soil health (Nkoa, 2014; Iqbal *et al.,* 2019; Iqbal, 2020a). Seaweed extract (SE) is a bio-organic fertilizer derived from large algae in the ocean (Ullah *et al.,* 2008). In this context, SE biostimulants are a greener alternative towards sustainably increasing crop yields (Ghosh *et al.,* 2015). Unlike the traditional organic inputs employed in rice production which usually result in increased environmental impacts, at least in the short term (Hokazono and Hayashi, 2012), these biostimulants are unique wherein stable yield improvements are observed when used along with conventional fertilizers. Furthermore, its contains several mineral components and biologically active substances that can benefit crops and soil (Adekiya *et al.,* 2019; Rathore *et al.,* 2009). Seaweed extract contains a variety of growth regulators, such as cytokinin, auxin, and gibberellin required for plant growth and development (Durand *et al.,* 2003). Seaweed extract can be naturally degraded, is non-toxic, does not pollute the environment, and provides an innocuous alternative to chemical fertilizers, which can induce much harm when applied in excess (Dhargalkar and Pereira, 2005).

Microbial agents are natural products composed of beneficial microorganisms and active microbial strains (Alori and Babalola, 2018). They can stimulate microbial activity directly or indirectly and improve soil nutrient mobility (Suyal *et al.,* 2016). They can be used as biological fertilizer in soil or plants and can promote plant growth, improve soil fertility, and enhance crop productivity (Jacoby *et al.,* 2017). Microbial agents are products containing specific living microorganisms that can decompose organic and inorganic substances in the soil, activate soil nutrients, and enhance the ability of plants to absorb mineral nutrients (Muhammad *et al.,* 2016). Application of MI can benefit the soil microbial composition and activity (Yu *et al.,* 2010; Liu *et al.,* 2015), may increase crop yield, improve soil chemical and physical properties including, the content and mineralization rate of soil organic matter, the contents of essential nutrients and the structure of soil aggregates (Zhang *et al.,* 2005; Liu *et al.,* 2015). Moreover, application of MI may offer an effective way for incorporation of large straw loads into cropping soils instead of burning, and has been increasingly adopted in recent years in China. The application of SE and microbial inoculants (MI) can promote an increase in rice biomass accumulation and rice production (Chen, 2017). Indeed, microbes are critical drivers of soil functions and agricultural crop productivity (Singh and Trivedi, 2017). One possible approach for reducing N input without compromising yield is to use a new green organic fertilizer as a supplementary fertilizer.

Rice leaf area index (LAI), dry matter accumulation (DMA), and panicle grain number are strongly associated with grain yield (Iqbal *et al.,* 2019). The LAI is an important indicator reflecting leaf coverage and crop growth (Katsura *et al.,* 2007). Some studies have suggested that increases in LAI are beneficial for increasing grain yield (Haboudane *et al.,* 2004; Hu *et al.,* 2019). DMA after the heading stage is also closely related to rice grain yield, and the yield is affected by the proportion of dry matter distributed to the ear and the amount of material transferred to the ear in the later stage (Tang *et al.,* 2015; Haiming *et al.,* 2018). Previous studies have focused on the effects of N application rate and fertilization timing on different rice cultivars. However, our understanding of how variation in N utilization efficiency affects the growth and biomass of different rice cultivars and its relationship to rice grain yield under combined organic and inorganic amendments is poor. Here, organic fertilizer combined with N fertilizer was hypothesized to improve soil functionality and thus increase leaf area, dry matter, and grain yield. The objectives of this study were twofold: i) to evaluate the joint effect of SE and MI with reduced N fertilizer on the growth, yield, and yield traits of different rice cultivars; and ii) to characterize the relationships between leaf physiological characteristics, biomass accumulation, and grain yield.

Materials and methods

Experimental site and climatic conditions

The experiment was conducted in Guangxi province, southern China at three different sites (*i.e*., Binyang County, Liucheng County, and Yulin City) during the early (March-July) and late seasons (August-November). Table 1 shows the soil chemical properties of the experimental sites. Soil samples were collected at the depth of 0-20 cm before the commencement experiment. The soil was air-dried and crushed for initial characterization of soil chemical properties. The soil is acidic in nature with a pH of 5.98, and having averaged soil organic carbon 19.35 (g kg^{-1}), total nitrogen 2.0 (g kg^{-1}), available phosphorous (188.78 mg kg^{-1}), and available potassium 140.66 (mg kg^{-1}).

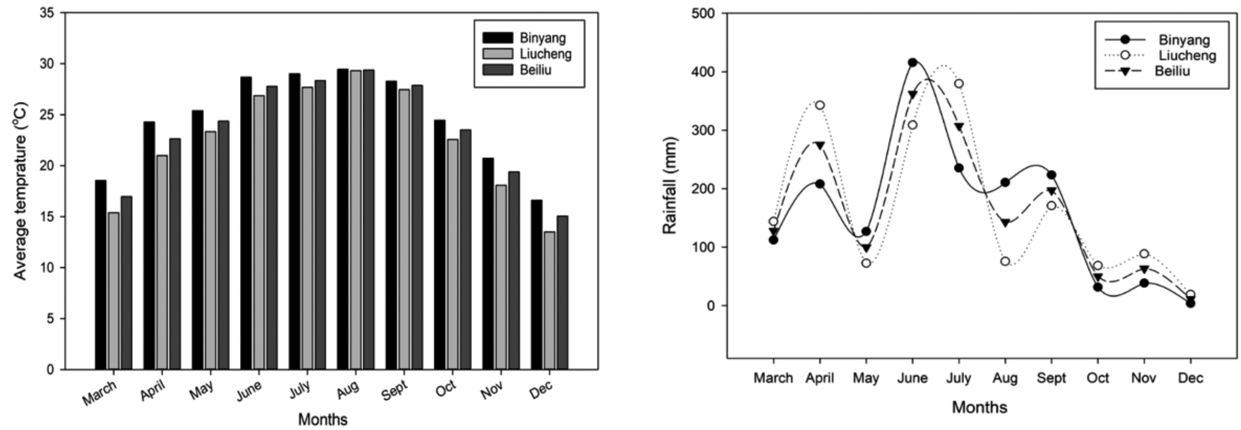

Figure 1. Average monthly temperature and precipitation (mm) of experimental sites (Binyang, Liucheng and Beiliu) during the early and late growing season. Note: Date collected from local weather monitoring station.

Table 1. Soil chemical properties before experimentation.

Site	pH	TN (g/kg)	SOC(g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)
Binyang	5.31	2.08	21.05	255.5	126.48	231
Lincheng	8.08	2.74	24.56	206.5	104.5	81
Yulin	4.97	1.2	12.45	108.5	335.36	110

TN, total nitrogen; SOC, soil organic carbon; AN, available nitrogen; AP, available phosphorous; AK, available potassium.

Treatment structure and field management

Field experiments were performed in a randomized complete block design with three replicates. The size of each plot was 20.2 m^2. Seaweed extract (SE) and microbial inoculants (MI) were the organic sources of fertilizer, and urea was the chemical N fertilizer used in this study. SE and MI are commercial products provided by Qingdao Haida biological Group Co., Ltd. The main components of SE: P_2O_5+K_20>20%, Organic matter >20%, seaweed polysaccharide >15%. Each bag of seaweed essence 500g. Furthermore, the microbial agent is a yellow fully soluble powder with an effective viable count of more than 20 billion/g. The study consisted of five treatments as reported in Table 2.

The recommended dose of phosphorous (P_2O_2) 90 (kg ha^{-1}) and potassium (K_2O) 180 (kg ha^{-1}) fertilizers were used in this study. Two different rice cultivars, Baixiang 139 (N efficient) and Guiyu 9 (N inefficient) were used as test crops. Initially, seeds were sown in the plastic trays; early rice was transplanted when seedlings were 25-days old, and late rice was transplanted when seedlings were 15 days old. Nitrogen (urea) and potassium fertilizers (KCL) were applied in three splits: 50% at transplanting, 30% at the tillering stage, and 20% at the panicle initiation stage. In contrast, phosphate fertilizer (P_2O_2), SE, and MI were applied as a basal fertilizer one day before seedling transplantation. Normal standing water was provided at a depth of four cm from transplantation to physiological maturity. All other agronomic practices (*i.e.*, irrigation, pesticides and insecticides) were conducted in the same manner for all treatments.

Table 2. Treatment combination.

Treatment	Nitrogen (kg ha^{-1})	SE (kg ha^{-1})	MI (kg ha^{-1})
T1 (CK)	180	0	0
T2	180	3	3
T3	144	3	3
T4	126	3	3
T5	108	3	3

SE, seaweed extract; MI, microbial inoculants.

Sampling and measurements

To determine DMA and LAI, five rice plants were randomly collected from each treatment at the heading and maturity stages. Rice plants were then divided into three parts: stems, leaves, and spikes. For LAI determination, 5 leaves from each plant were randomly selected, and leaf length and width were measured with a ruler. LAI was calculated by the specific leaf weight method according to the method of Tiansheng *et al.* (2007). The rice samples were dried in an oven with starting temperature of 105°C for 30 min and continued the drying process at 85°C for 72 h for determination of DMA. After rice harvesting and threshing, the samples dried with sunlight until 14% moisture content was achieved, measurements of grain yield were taken (Figure 1).

Data analysis

Analysis of variance was conducted to test the differences in growth, dry matter accumulatio, grain yield and yield attributes of rice using Statistics 8.1 analytical software. The collected data were first check for normal distribution and after following the assumptions. Data were analysed in a completely randomized design using one-way ANOVA. Microsoft Excel 2010 was used to organize the data, and data in percentages were arcsine-transformed to normalize the variables before analysis. Means were differentiated using the least significant difference tests at $P<0.05$. Pearson's correlation analyses were conducted to evaluate the relationships between growth, yield, and yield components in Statistics 8.1 software.

Results

Leaf area index

Leaf area index was significantly affected by treatment, cultivar, and site during both seasons (Table 3). During the early season, the highest LAI (3.45 cm^2) was observed in T_3 of Baixiang 139, whereas the lowest (2.09 cm^2) LAI was observed in T_5 at Binyang site. During the late season, no significant differences in LAI ($P<0.05$) were observed at Binyang site. There were significant differences in the LAI of Baixiang 139 and Guiyu 9 under each fertilization treatment during the early season at Liucheng site. Baixiang 139 had the highest LAI (2.78 cm^2) in T_2.

There were no significant differences in the LAI of Baixiang 139 and Guiyu 9 under each fertilization treatment in the early season at Yulin. There were no significant differences in the LAI of Baixiang 139 under each fertilization treatment at Liucheng and Yulin sites. Furthermore, there were no significant differences ($P<0.05$) in the LAI during the late season under each treatment at Binyang site. However, the LAI of Baixiang 139 under each treatment was significantly different at Liucheng site. The Baixing 139, cultivar had the highest LAI (2.72 cm^2) in T_2, and the lowest LAI (1.49 cm^2) was observed in T_4. There were no significant differences in LAI across treatments at Yulin.

Dry matter accumulation

Dry matter accumulation at the heading stage

The combined application of SE and MI with reduced N rates significantly affected DMA at the heading stage at Liucheng in the early season; no significant differences were observed among the other test sites. There were no significant differences in the DMA of Baixiang 139 and Guiyu 9 under each fertilization treatment (Table 4). However, differences in DMA among the different sites for Baixiang 139 and Guiyu 9 were statistically ($P<0.05$) significant under each fertilization treatment (Table 3). Baixiang 139 produced the highest DMA (6723 kg ha^{-1}) under T_2, and the lowest DMA (5010 kg ha^{-1}) was observed in T_5. However, the DMA of T_3 was not significantly different ($P<0.05$) from that of T_2. Similarly, Guiyu 9 led to the highest DMA (7786 kg ha^{-1}) in T_2 and the lowest DMA (96086 kg ha^{-1}) in T_5. In addition, there were no significant differences in the DMA of Baixiang 139 and Guiyu 9 under each fertilization treatment at Yulin site and the other test sites.

Dry matter accumulation at the maturity stage

The co-application of SE and MI with reduced N rates significantly affected the DMA at the maturity stage at Liucheng site; however, no significant differences were observed among the other test sites. There were no significant differences in the DMA of Baixiang 139 and Guiyu 9 under each fertilization treatment (Table 4). The N-efficient cultivar Baixiang 139 resulted in the highest DMA (11,196 kg ha^{-1}) under T_2. Similarly, the N-inefficient cultivar Guiyu 9 produced the highest DMA (12,497 kg ha^{-1}) in T_2 and the lowest DMA (10,248 kg ha^{-1}) in T_4. However, there were no significant differences in the DMA of Baixiang 139 and Guiyu 9 under each fertilization treatment at Yulin and Binyang sites. There were also no significant differences in the DMA of Baixiang 139 under each fertilization treatment at Liucheng site; however, there were substantial differences in the DMA of Guiyu No 9 at Liucheng. The DMA of Guiyu 9 was highest (99,819 kg ha^{-1}) in T_2 and lowest (8165 kg ha^{-1}) in T_4. There were no significant differences in the DMA of Baixiang 139 and Guiyu 9 under each fertilization treatment at Yulin site.

Rice yield and yield components

Rice yield and yield components (*i.e.*, panicle number, grains per panicle, and grain yield) were significantly affected by N level coupled with SE and MI ($P\leq0.05$) (Table 5). The productive panicle number of Baixiang 139 and Guiyu 9 at Liucheng significantly differed among treatments. However, there were no significant differences in the grain filling rate (%) and 1000-grain weight (g, TGW) among experimental sites and cultivars. The combined fertilization of SE and MI with lower N rates significantly increased rice grain yield at different sites (Table 5). During the early season, the rice grain yield of Baixiang 139 was 5.28% and 3.44% higher in T_2 and T_3, respectively, compared with the control. The rice grain yield of Guiyu 9 was 6.74% higher in T_3 compared with the control. During the late season, the grain yield of Baixiang 139 was 2.19% and 1.04% higher in T_2 and T_3, respectively, compared with the control. The grain yield of Guiyu 9 was 1.91% and 1.35% high-

Table 3. Effect of combined seaweed and microbial inoculants on rice leaf area index.

Cultivars	Trt	Early (cm^2)			Mean	Late (cm^2)			Mean
		Binyang	Liucheng	Yulin		Binyang	Liucheng	YuLin	
Baixiang- 139	T_1	3.45 ± 0.79^{a}	2.67 ± 0.47^{a}	2.94 ± 0.26^{a}	3.02	1.35 ± 0.56^{a}	1.97 ± 0.45^{bc}	2.55 ± 0.17^{a}	1.96
	T_2	2.98 ± 0.14^{ab}	2.78 ± 0.27^{a}	3.42 ± 0.83^{a}	3.06	1.36 ± 0.03^{a}	2.72 ± 0.45^{a}	2.51 ± 0.48^{a}	2.20
	T_3	2.09 ± 0.06^{b}	1.84 ± 0.05^{c}	2.67 ± 0.28^{a}	2.20	1.21 ± 0.25^{a}	2.17 ± 0.43^{ab}	2.28 ± 0.38^{a}	1.89
	T_4	2.79 ± 0.54^{ab}	2.27 ± 0.33^{b}	2.64 ± 0.61^{a}	2.57	1.18 ± 0.39^{a}	1.49 ± 0.16^{c}	2.80 ± 0.52^{a}	1.82
	T_5	2.90 ± 1.03^{ab}	1.89 ± 0.17^{bc}	2.25 ± 0.83^{a}	2.35	1.30 ± 0.1^{a}	1.79 ± 0.42^{bc}	2.63 ± 0.37^{a}	1.91
Guiyu-9	T_1	4.06 ± 0.35^{a}	3.25 ± 0.33^{a}	4.04 ± 1.03^{a}	3.78	0.72 ± 0.22^{a}	2.71 ± 0.19^{a}	2.35 ± 0.67^{a}	1.93
	T_2	4.48 ± 0.43^{a}	3.04 ± 0.05^{ab}	2.98 ± 0.72^{a}	3.50	0.90 ± 0.13^{a}	2.85 ± 0.63^{a}	2.38 ± 0.73^{a}	2.04
	T_3	3.63 ± 0.69^{a}	2.51 ± 0.48^{bc}	3.91 ± 0.88^{a}	3.35	1.14 ± 0.52^{a}	2.51 ± 0.32^{a}	2.64 ± 1.05^{a}	2.10
	T_4	3.89 ± 0.56^{a}	2.48 ± 0.44^{bc}	4.28 ± 0.51^{a}	3.55	0.91 ± 0.15^{a}	2.18 ± 0.38^{a}	2.07 ± 0.67^{a}	1.72
	T_5	4.21 ± 0.27^{a}	1.99 ± 0.08^{c}	3.03 ± 0.66^{a}	3.08	1.36 ± 0.5^{a}	2.08 ± 0.51^{a}	1.97 ± 0.32^{a}	1.80

T_1, N180 + SE0 + MI0 (kg ha^{-1}); T_2, N180 + SE3 + MI3 (kg ha^{-1}); T_3, N144 + SE3+MI3 (kg ha^{-1}); T_4, N126 + S3kg ha + MI3 (kg ha^{-1}); T_5, N108 + SE3 + MI3 (kg ha^{-1}). SE, seaweed extract; MI, microbial inoculants; Trt, Treatment. [a-c]Values followed by the same letters within column are not significantly different at $P<0.05$.

er in T_2 and T_3, respectively, compared with the control. However, there were no significant differences in grain yield among the different sites. Table 5 shows that the average grain yield of the early and late seasons at the three test sites was significantly affected by fertilization treatment. The grain yield of Baixiang 139 was 3.75%, and 2.25% higher in T_2 and T_3, respectively, compared with the control. The grain yield of Guiyu 9 was 1.34% and 4.28% higher in T_2 and T_3, respectively, compared with the control.

Table 4. Effect of combined seaweed and microbial inoculants on rice dry matter accumulation.

Cultivars		Trt	Early season (kg ha^{-1})			Mean	Late season (kg ha^{-1})			Mean
			Binyang	Liucheng	Yulin		Binyang	Liucheng	YuLin	
Heading stage	Baixiang 139	T_1	5407±915^{a}	6213±132^{ab}	7348±163^{a}	6323	6778±583^{a}	8249±209^{a}	7484±1139^{a}	7504
		T_2	6115±574^{a}	6724±867^{a}	6869±77^{ab}	6570	6577±135^{a}	8440±300^{a}	7149±1257^{a}	7389
		T_3	5615±1018^{a}	5801±1067^{ab}	6497±516^{b}	5971	6290±1812^{a}	8430±536^{a}	6678±236^{a}	7132
		T_4	5849±1201^{a}	5270±774^{ab}	6307±606^{b}	5809	5862±586^{a}	8595±1063^{a}	7480±416^{a}	7312
		T_5	5103±1166^{a}	5011±649^{b}	7121±643^{ab}	5745	6185±36^{a}	8709±224^{a}	6959±877^{a}	7284
	Guiyu 9	T_1	7498±498^{a}	7664±670^{ab}	8968±960^{a}	8043	5742±899^{a}	9156±1315^{ab}	7490±1158^{a}	7463
		T_2	7956±205^{a}	7786±532^{a}	9939±708^{a}	8560	6365±802^{a}	9819±841^{a}	7520±1238^{a}	7901
		T_3	7331±1224^{a}	6743±112^{bc}	9944±669^{a}	8006	7436±1714^{a}	8978±503^{ab}	6829±808^{a}	7747
		T_4	6600±1492^{a}	6104±472^{c}	9659±1671^{a}	7454	6549±1899^{a}	8165±648^{b}	7301±545^{a}	7339
		T_5	6433±609^{a}	6087±411^{c}	8456±122^{a}	6992	6115±1387^{a}	8279±432^{ab}	7321±232^{a}	7238
Maturity stage	Baixiang 139	T_1	9589±1182^{a}	9864±478^{ab}	11762±1424^{ab}	10405	8004±2280^{a}	10229±1631^{a}	10387±771^{a}	9540
		T_2	11006±2822^{a}	11196±1263^{a}	14050±2281^{a}	12084	8254±894^{a}	12080±1899^{a}	10847±1002^{a}	10393
		T_3	8218±416^{a}	9766±904^{ab}	11314±729^{b}	9766	8654±2752^{a}	10285±740^{a}	10570±487^{a}	9836
		T_4	8182±1823^{a}	9327±536^{b}	10377±61^{b}	9295	7530±1411^{a}	10077±481^{a}	10607±1846^{a}	9405
		T_5	8596±2314^{a}	9466±177^{b}	10313±1363^{b}	9458	8467±380^{a}	10737±1426^{a}	10531±1383^{a}	9916
	Guiyu 9	T_1	11003±591^{a}	13284±905^{a}	11317±126^{a}	11868	7186±385^{a}	11365±394^{a}	10015±2972^{a}	9522
		T_2	12929±1965^{a}	12497±689^{a}	12942±1497^{a}	12790	6136±194^{a}	11748±917^{a}	9788±1005^{a}	9224
		T_3	11304±2839^{a}	11599±1373^{ab}	16146±5820^{a}	13016	7797±1672^{a}	11946±737^{a}	9649±1571^{a}	9797
		T_4	11660±1213^{a}	10249±1035^{b}	14851±2206^{a}	12253	6375±417^{a}	10390±1245^{a}	8765±254^{a}	8510
		T_5	12389±1364^{a}	10420±603^{b}	11854±1532^{a}	11554	7354±832^{a}	10871±858^{a}	9129±912^{a}	9118

$^{a-c}$Values followed by the same letters within column are not significantly different at P<0.05.

Table 5. Effect of combined seaweed and microbial inoculants on early rice plant productive tillers, grain per panicle, filled grain rate, grain weight and grain yield.

Site	Cultivars	Treatment	PF (10^{4}/ha)	GP	FGR%	1000-GW (g)	GY (kg ha^{-1})
Binyang	Baixiang 139	T_1	306±34^{a}	120±2.5^{ab}	77.78±7.3^{a}	18.07±0.43^{a}	4737±331.48^{ab}
		T_2	338±59^{a}	117±8.3^{ab}	84.27±4.8^{a}	18.16±0.41^{a}	5255±173^{a}
		T_3	313±39^{a}	102±1.5^{b}	80.56±7.5^{a}	18.31±0.46^{a}	4881±546^{ab}
		T_4	213±41^{b}	147±43^{a}	82.55±4.5^{a}	18.31±0.5^{a}	4284±589^{b}
		T_5	256±34^{ab}	118±15^{ab}	85.91±1.7^{a}	17.82±0.53^{a}	4516±412^{ab}
	Guiyu 9	T_1	283±27^{a}	110±1^{b}	68.84±1.2^{b}	22.46±0.38^{a}	4691±256^{b}
		T_2	292±21^{a}	119±10^{ab}	73.85±1.4^{a}	22.52±1.03^{a}	5077±144^{ab}
		T_3	251±63^{a}	131±6.1^{a}	70.45±3.5^{ab}	22.84±0.7^{a}	5542±817^{a}
		T_4	289±44^{a}	114±8.2^{ab}	69.84±3.1^{ab}	22.56±0.23^{a}	4782±231^{ab}
		T_5	268±12^{a}	119±16^{ab}	72.48±2.0^{ab}	22.81±0.55^{a}	4673±315^{b}
Liucheng	Baixiang 139	T_1	257±16^{a}	133±17^{a}	70.21±7.4^{a}	17.72±0.43^{a}	5088±138^{a}
		T_2	281±25^{a}	142±17^{a}	76.85±6.5^{a}	17.77±0.28^{a}	5362±123^{a}
		T_3	246±29^{a}	131±22^{a}	74.39±3.2^{a}	17.31±0.12^{a}	5179±116^{a}
		T_4	254±19^{a}	138±4.6^{a}	76.58±2.9^{a}	17.33±0.16^{a}	5098±200^{a}
		T_5	237±3^{a}	148±12^{a}	67.38±13.2^{a}	17.31±0.14^{a}	4681±296^{b}
	Guiyu 9	T_1	240±5^{a}	184±3.7^{a}	59.76±5.4^{a}	20.55±0.26^{a}	5637±116^{a}
		T_2	233±17^{a}	179±24^{a}	61.14±3.7^{a}	20.25±1.29^{a}	5403±81^{ab}
		T_3	224±13^{ab}	161±34^{a}	65.57±7.3^{a}	21.17±0.78^{a}	5535±336^{ab}
		T_4	206±7^{bc}	160±5.9^{a}	66.75±2.5^{a}	21.19±0.67^{a}	5149±247^{bc}
		T_5	198±15^{c}	175±3.7^{a}	63.12±6.0^{a}	20.74±0.25^{a}	4894±116^{c}
Yulin	Baixiang 139	T_1	404±42^{a}	104±8.2^{a}	77.56±6.5^{a}	17.59±0.53^{a}	5320±745^{b}
		T_2	399±32^{a}	120±4.8^{a}	80.28±1.8^{a}	17.72±0.14^{a}	6269±304^{a}
		T_3	349±53^{a}	120±17^{a}	74.72±1.7^{a}	17.49±0.2^{a}	5641±271^{ab}
		T_4	375±20^{a}	112±14^{a}	71.89±14^{a}	18.02±0.83^{a}	5471±268^{ab}
		T_5	367±37^{a}	113±11^{a}	77.96±1.2^{a}	17.68±0.42^{a}	5220±385^{b}
	Guiyu 9	T_1	295±18^{a}	109±5.9^{a}	43.54±6.5^{a}	21.43±0.43^{a}	4768±494^{a}
		T_2	293±41^{a}	130±47^{a}	58.95±1.0^{a}	21.09±1.67^{a}	4746±229^{a}
		T_3	330±31^{a}	138±49^{a}	54.63±2.0^{a}	21.67±0.51^{a}	5035±479^{a}
		T_4	327±9^{a}	125±28^{a}	54.29±1.2^{a}	22.05±0.76^{a}	5006±343^{a}
		T_5	277±44^{a}	113±17^{a}	49.71±15^{a}	22.25±0.16^{a}	4950±271^{a}

PF, productive panicle; GP, grain per panicle; FGR, filled grain rate; GW, grain weight; GY, grain yield. $^{a-c}$Values followed by the same letters within column are not significantly different at P<0.05.

The effect of different nitrogen levels on actual rice yield

There were no significant differences in actual yield among the various fertilization treatments. During the early season, the actual yield of Baixiang 139 was 5.28% and 3.44% higher in T_2 and T_3, respectively, compared with the control (Table 5). Similarly, the actual yield of Guiyu 9 was 6.74% and 0.86% higher in T_3 and T_2, respectively, compared with the control. The actual yield of Guiyu 9 was 1.91% and 1.35% higher in T_2 and T_3, respectively, compared with the control. However, across both seasons, T_2 increased the grain yield by 2.57% compared with the control.

Correlation analysis between rice agronomic traits and actual yield under different nitrogen levels

The correlation analysis of the relevant indicators under the combined fertilization is shown in Tables 6 and 7. The correlation analysis revealed that the LAI, DMA, effective spike, total grains per spike, and grain filling rate were positively correlated with the grain yield of rice. This analysis showed that increases in growth and yield components significantly contributed to the higher grain yield of rice.

Table 6. Effect of combined seaweed and microbial inoculants on late rice plant productive tillers, grain per panicle, filled grain rate, grain weight and grain yield.

Site	Cultivars	Treatment	PF (104/ha)	GP	FGR%	1000-GW (g)	GY (kg ha–1)
Binyang	Baixiang 139	T_1	293±95^{a}	105±7.1^{a}	58.14±5.9^{a}	19.09±1.6^{a}	3449±646^{a}
		T_2	273±52^{a}	108±9.2^{a}	64.18±4.3^{a}	18.20±1.09^{a}	3215±559^{a}
		T_3	276±74^{a}	112±2.2^{a}	61.10±2.5^{a}	19.05±0.93^{a}	3592±249^{a}
		T_4	269±59^{a}	111±4.3^{a}	64.82±10^{a}	18.31±0.2^{a}	3215±538^{a}
		T_5	300±23^{a}	112±10^{a}	60.60±3.8^{a}	18.12±0.7^{a}	2829±187^{a}
	Guiyu 9	T_1	153±11^{a}	149±8.9^{a}	53.91±9.8^{a}	22.05±1.12^{a}	2483±474^{a}
		T_2	133±16^{a}	135±35^{a}	56.60±13^{a}	22.53±2.67^{a}	2564±500^{a}
		T_3	136±8.8^{a}	165±14^{a}	50.42±5.8^{a}	23.08±0.69^{a}	2584±336^{a}
		T_4	138±25^{a}	149±17^{a}	51.64±4.9^{a}	22.66±0.72^{a}	2350±294^{a}
		T_5	137±36^{a}	198±70^{a}	55.10±6.4^{a}	22.09±1.35^{a}	2422±708^{a}
Liucheng	Baixiang 139	T_1	290±22^{b}	151±25^{a}	48.70±1.6^{a}	19.62±0.45^{a}	5482±544^{a}
		T_2	335±15^{a}	155±27^{a}	53.50±5.7^{a}	18.86±1.12^{a}	5659±106^{a}
		T_3	289±16^{b}	139±19^{a}	56.46±11.5^{a}	19.24±0.14^{a}	5534±403^{a}
		T_4	265±22^{b}	181±39^{a}	51.12±8.7^{a}	18.80±1.06^{a}	5129±74^{a}
		T_5	280±16^{b}	161±12^{a}	55.34±4.6^{a}	19.15±0.58^{a}	5271±400^{a}
	Guiyu 9	T_1	215±19^{b}	159±9.6^{ab}	53.16±3.0^{a}	22.61±0.79^{ab}	5415±755^{a}
		T_2	254±28^{a}	153±19^{b}	52.11±3.3^{a}	21.87±0.72^{b}	5364±107^{a}
		T_3	214±12^{b}	194±21^{a}	52.82±3.7^{a}	22.48±0.26^{ab}	5374±217^{a}
		T_4	209±13^{b}	159±30^{ab}	50.50±4.4^{a}	23.13±0.55^{a}	5016±663^{a}
		T_5	209±4.8^{b}	167±11^{ab}	53.62±2.2^{a}	22.88±0.25^{ab}	4861±222^{a}
Yulin	Baixiang 139	T_1	263±15^{a}	156±41^{a}	74.74±9.9^{a}	19.02±0.36^{a}	5343±249^{a}
		T_2	269±48^{a}	139±16^{a}	71.88±0.5^{a}	19.08±0.36^{a}	5712±739^{a}
		T_3	250±33^{a}	128±12^{a}	74.70±4.5^{a}	18.75±0.68^{a}	5297±457^{a}
		T4	257±29^{a}	144±15^{a}	71.22±4.8^{a}	18.42±0.34^{a}	5103±518^{a}
		T_5	247±6.7^{a}	142±27^{a}	74.82±6.8^{a}	19.13±0.47^{a}	4799±269^{a}
	Guiyu 9	T_1	176±16^{a}	180±71^{a}	58.37±2.5^{a}	23.23±1.43^{a}	4790±385^{a}
		T_2	182±22^{a}	168±19^{a}	55.10±2.3^{a}	24.05±0.91^{a}	5002±419^{a}
		T_3	186±26^{a}	160±25^{a}	63.73±7.6^{a}	23.17±0.24^{a}	4900±96^{a}
		T_4	164±5^{a}	169±4^{a}	64.76±12^{a}	22.40±0.35^{a}	4827±304^{a}
		T_5	166±7.5^{a}	165±21^{a}	65.11±8.5^{a}	23.51±1.27^{a}	4780±488^{a}

PF, productive panicle; GP, grain per panicle; FGR, filled grain rate; GW, grain weight; GY, grain yield. ^{a,b}Values followed by the same letters within column are not significantly different at $P<0.05$.

Table 7. Correlation analysis between grain yield and yield components of rice.

Index	LAI	GY	DM	PT	GP	FGR	TGW
LAI	1						
GY	0.69*						
DM	0.62*	0.79*					
PT	0.74*	0.48	0.73*				
GP	–0.37	0.19	0.05	–0.57			
FGR	0.60*	0.23	–0.08	0.17	–0.39		
TGW	–0.28	–0.19	–0.14	–0.31	0.08	–0.3	1

LAI, leaf area index; GY, grain yield; DM, dry matter; PT, productive tillers; GP, grain per spike; TGW, 1000-grain weight; FGR, filled grain rate. *P=0.05.

Discussion

The appropriate use of N fertilizer is key for meeting the demand of the growing human population for food and soil for improving soil health and cereals crop quality (Iqbal *et al.,* 2020b; Izhar *et al.,* 2020; Ullah *et al.,* 2020). However, the overuse of chemical N fertilizer can lead to several problems, such as environmental pollution, soil degradation, water contamination, and decreased crop productivity (Peng *et al.,* 2015; Cai *et al.,* 2018). Bio-organic fertilizers such as SE and MI provide viable alternatives to chemical fertilizer, as they do not have the negative effects of synthetic fertilizer and can promote sustainable agricultural production (Babalola and Glick, 2012). The goal of this study was to assess whether sustainable improvements in rice growth and production could be achieved by reducing the amount of synthetic N fertilizer applied and adding organic fertilizer, such as SE and MI).

The combined fertilization of N with SE and MI at two rates [N 180 + SE 3 + MI 3 (kg ha^{-1})] and [N 144 + SE 3 + MI 3 (kg ha^{-1})] significantly increased the LAI, DMA, productive panicle number, grains per panicle, grain filling rate, TGW, and grain yield of rice compared with the control. The increases in growth, yield, and yield components were attributed to the combined fertilization of synthetic and bio-organic fertilizers in this study. These findings can be explained by the fact that SE and MI improve soil health and quality, which enhances plant nutrient uptake and thereby increases plant growth and biomass accumulation (Ullah *et al.,* 2008; Jacoby *et al.,* 2017). Furthermore, increases in growth and yield traits under combined treatments might stem from the presence of several cytokinin enzymes, including trans-zeatin riboside and their dihydro derivatives, which improve plant growth and health (Begum *et al.,* 2018). The bioactive compounds in *Ascophyllum nodosum* extract and its organic subfractions have been shown to affect legume–rhizobia signalling mechanisms and result in more functional nodules and overall improvements in the growth of plants (Khan *et al.,* 2013). Consistent with our findings, the application of SE has been shown to enhance early plant development and lead to increases in yield components in legume plants, including 12-25% higher grain yield, compared with the control (Sethi and Adhikary, 2008).

Another study found that the yield of the bean was improved by 25% using a foliar spray of SE (Haider *et al.,* 2012). Sarhan *et al.* (2011) reported that SE can have positive effects on the growth and development of potatoes, and significantly improve the yield and quality of potatoes. The application of SE can also improve the amount of micronutrients in rice grains, such as Cu and Zn, by up to 10% and Fe and Mn by up to 5% (Layek *et al.,* 2014). SE contains chelating compounds, such as mannitol, that can increase the accessibility of some micronutrients to plants (Shah *et al.,* 2013). Similar findings have also been made in wheat and okra (Zodape *et al.,* 2009; Shah *et al.,* 2013). The spraying of SE at 30 and 60-day intervals after planting has been shown to maximize tuber yield, enhance N uptake, and increase protein content in potatoes (Haider *et al.,* 2012).

In our study, increases in grain yield under the co-inoculation of SE and MI could stem from its positive effects on root proliferation and the uptake of N, P, and sulphur (S), which are required for protein synthesis (Shah *et al.,* 2013). In addition, MI also improve plant growth by encouraging root development and altering root architecture through the processing of phytohormones, such as indole-3-acetic acid (Alori *et al.,* 2017), which leads to increases in the number of root tips, surface area, and root length (Gamalero *et al.,* 2002; Vacheron *et al.,* 2013). Such root stimulation can help protect plants from pathogens and may also be linked to the induction of systemic tolerance (Ramirez and Maiti*,* 2016). These improvements in root morphological and physiological traits enhance root nutrient uptake capacity, which in turn increases N use efficiency, DMA, and grain yield (Yang *et al.,* 2009; Izhar *et al.,* 2020). A previous study found that *Pseudomonas fluorescens* had a positive effect on the growth of mung bean (*Vigna radiata*) plants *in vitro* and *in situ* by promoting increases in the root (30% and 20%) and shoot length (20% and 24%) through the P solubilization associated with acid production, which led to a decrease in pH (Katiyar and Goel*,* 2003). Botelho *et al.* (2015) reported a similar finding: the BR-5 strain of *P. fluorescens* stimulated the growth of maize in natural soil. MI increases plant growth and crop yield as well as a host plant nutrient. Other researchers, such as Arthur *et al.* (2003) and Zodape *et al.* (2008) have noted a substantial increase in the grain yield of crops stemming from the combined use of SE and MI. Furthermore, SE foliar spray could provide a promising alternative for improving yields under rainfed soybean production, as SE has been found to substantially enhance the oil content, oil yield, K, N, and raw sunflower seed proteins (Rathore *et al.,* 2009; Osman and Salem*,* 2011).

Conclusions

Leaf area index, biomass accumulation, productive grains per panicle, grain filling rate, 1000-grains weight, and grain yield of rice were increased under the combined application of nitrogen fertilizer with seaweed extraction and microbial inoculants compared with the sole application of chemical fertilizer. Data from the three different experimental sites showed that the rice cultivars Baixiang 139 and Guiyu 9 can maintain higher growth yield and yield traits under the co-application treatment [N 144 + SE 3 + MI 3 (kg ha^{-1})]. Furthermore, the correlation analysis revealed that the increases in the growth and yield traits significantly contributed to the higher grain yield of rice. Thus, the combined use of seaweed extraction and microbial inoculants with chemical fertilizer application improved the growth and yield of rice despite a 20% reduction in the application of synthetic nitrogen fertilizer.

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