Research Article

Monitoring soil microorganisms with community-level physiological profiles using Biolog EcoPlates™ in Chaohu lakeside wetland, east China

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Abstract

Under the circumstance of wetland degradation, we used Biolog EcoPlates™ method to investigate the impact of ecological restoration on the function of topsoil microbial communities by monitoring their metabolic diversity around Chaohu lakeside wetland. Four restoration patterns including reed shoaly land (RL), poplar plantation land (PL), abandoned shoaly grassland (GL) and cultivated flower land (FL) were selected. The result showed a rapid growth trend at the initial stage of incubation, following the fastest change rate at 72 h in both dormant and growing seasons, and the AWCD values of RL pattern was the highest at the detection points of each culture time, while the GL were the lowest. The calculation of diversity indicators also displayed significant lower McIntosh index in dormant season and Shannon-Wiener index in growing season in GL than in the others ($P < 0.05$). Carbohydrates and carboxylic acids were found to be the dominant substrates used in dormant season, whereas amino acids, polymers and phenolic acids were increasingly utilized by the microbial communities in growing season. We observed soil total potassium as the key factor that significantly affected the utilization efficiency of different carbon sources in both seasons ($P < 0.05$).

Introduction

As one part of the terrestrial carbon pool, wetlands play a crucial role in global carbon cycling process [1]. Soil is the main component of the wetland ecosystem, and it can be strongly impacted by the hydrological changes caused by alternation of wetting

and drying, which alter the edaphic redox environment and thus control biogeochemical processes [2]. With the development of urbanization, wetland environment has been degraded resulted from agricultural reclamation, global climate change and acid precipitation [3-5], which makes wetland become one of the most threatened ecosystems in the world [6]. Nowadays people's awareness of ecological and environmental protection has been raised, and different restoration methods have been implemented to improve the wetland soil quality and species diversity. However, wetland carbon sequestration is still threatened by global warming [7]. It was reported that exogenous heavy metal inputs from agricultural development are introduced into natural wetlands [8, 9], which may significantly affect carbon cycling and balance of the area.

In a whole wetland ecosystem, microorganisms, as the decomposer and basic agent of the element circulation, play a key role in soil formation process [10, 11]. To make scienti fic researches in the reconstruction and restoration of urban lakeside wetland, it is necessary to explore soil microbial communities that affecting nutrient cycling and functional metabolism, especially for the utilization of carbon substrates. Although microorganisms are the important component for functioning of wetland ecosystem, their metabolic versatilities are poorly understood due to the complicated environmental stresses and regional microbial differentiation [12]. The Biolog EcoPlates™ is a method that relatively easy to operate, which is generally used to describe the diversity of community-level physiological profiles (CLPPs) [13-15]. Based on the biological and biochemical properties, the Biolog EcoPlates™ method

61 can quickly characterize the ecological status of environmental samples [16], such as
62 activated sludge [17], wastewater [18], sediments [19], and soils [13, 20].

63 As the fifth largest freshwater lake in China, Chaohu Lake wetland has been
64 suffered from long term interference and damage due to the irrational use and excessive
65 exploitation of resources in agricultural and construction activities [21-23]. Luckily, the
66 environmental problems resulting from artificial disturbance has raised widely concern
67 to the public. Native vegetation was basically non-existent, whereas since the year of
68 2003, large portions of the reclaimed land have been gradually restored to wetland and
69 artificial forest [24, 25]. This is an extensively employed ecological restoration
70 technique that plays a vital role in the Chaohu Lake watershed management [26].
71 Recent efforts have been done on the eutrophication of water ecosystem about Chaohu
72 Lake [23, 27], however, there were little researches analyzing the functional
73 metabolism shifts of microbial communities during the ecological restoration in this
74 wetland ecosystem.

75 The objective of the present study was to investigate whether and how ecological
76 restoration affect the wetland microbial community ecological functions along the
77 Chaohu lakeside wetland. Based on the Biolog method, we further intended to find the
78 specific metabolic characteristics and seasonal shifts of topsoil microbial communities
79 in different patterns, which may help to estimate the efficiency of various restoration
80 ways from the perspective of functional diversity of soil microorganisms.

81 # Materials and methods

Site Description

This study was conducted around the northwest Chaohu Lake wetland between Pai River and Nanfei River, approximately 15 km from southern Hefei City, Anhui Province, China (30°25′28″N~31°43′28″N, 117°16′54″E~117°51′46″E). The area is influenced by the subtropical humid monsoon climate, and there are more than 230 days frost-free period, with the annual mean temperature and precipitation of 15-16℃ and 900-1100 mm, respectively. The coastal soil types of Chaohu Lake and its main rivers belong to paddy soil.

Sampling Design and Field Measurement

Four types of wetland soils were selected representing different restoration patterns, including reed shoaly land (RL, natural restoration pattern), poplar plantation land (PL, artificial restoration pattern), abandoned shoaly grassland (GL, Control) and cultivated flower land (FL, artificial restoration pattern). PL site was located at the Hefei Lakeside Wetland Forest Park, FL and GL sites were chosen from the Dawei Eco-agricultural Park, and RL site was selected randomly along the basin area, with specific marks on the sampling sites. Field measurement was implemented in the vegetation dormant and growing season (early March and late July 2018). Basic information and vegetation characteristics of the experimental sites were shown in Table 1.

Table 1. Basic characteristics of experimental sites in Chaohu lakeside wetland

Pattern	Latitude	Longitude	Dominant vegetation			Hydrological condition	Underground water level（cm）
			Species	Family	Genus		
GL	31°40'43"	117°17'37"	*Digitaria sanguinalis*	Poaceae	*Digitaria*	Seasonal flooding	80
RL	31°43'14"	117°21'58"	*Phragmites australis*	Poaceae	*Phragmites*	Seasonal flooding	70
PL	31°43'27"	117°23'22"	*Populus deltoides* cv.'I-69'; *Populus euramevicana* cv.'I-214'	Salicaceae	*Populus*	Seasonal flooding	100
FL	31°39'55"	117°15'52"	*Zinnia elegans* Jacq.	Compositae	*Zinnia*	Intermittent flooding	80

After removing the litter layer, we collected several soil subsamples using a soil auger (6 cm in diameter), mixed together as one sample in each plot, and three plots were selected for biological replicates of each pattern. The samples were stored into sealed polyethylene bags and transported to the laboratory in a cooler box with ice bags. After passed through a 2 mm sieve to remove roots and other debris, the samples were divided into two equal parts. One part was for Biolog EcoPlates™ analysis, another for

111 soil physiochemical property measurement, including soil water content (SWC), pH,
112 dissolved organic carbon (DOC), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^-
113 -N), soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP) and total
114 potassium (TK) contents.

115 Soil characteristics analysis

116 SWC was measured by oven-drying at 105 °C to a constant weight. Soil pH was
117 determined using a pH meter in 1:2.5 soil / water suspensions. NH_4^+-N and NO_3^--N
118 were measured by a flow injection auto-analyzer (FIAStar 5000, FOSS, Sweden), as
119 well as TP after micro-Kjeldahl digestion. SOC and TN were measured with a CN
120 Analyzer (EA 3000, Vector, Italy). DOC was determined using a TOC auto-analyzer
121 (Multi N/C 3100, Jena Analytik, Germany) [28]. In detail, one part samples (30 g fresh
122 soil) were extracted with 50 mL of 0.5 mol·L^{-1} K_2SO_4 solution, shaking for 30 minutes
123 and then filtered. The filtrate was diluted and determined by the TOC instrument. TK
124 was measured by the Atomic Absorption Spectrophotometer (TAS-990AFG, Pgeneral,
125 China) after micro-Kjeldahl digestion.

126 Community Level Physiological Profiling

127 The Biolog EcoPlates™ method was used to conduct a 7-day dynamic monitoring
128 on the functional diversity of soil microbial community, and the average well color
129 development (AWCD) values of carbon-source utilization were collected [29]. Every
130 EcoPlate had 96 wells containing 31 different carbon sources plus a blank well, in three
131 replications. The utilization rate was pointed by the reduction of tetrazolium violet

132 redox dye, which changed from colorless to purple if the single source was used by
133 added microorganisms [16]. In addition, EcoPlate substrates were subdivided into six
134 groups: amino acids, amines, carbohydrates, carboxylic acids, phenolic acids and
135 polymers [30].

136 The EcoPlates to be tested were prepared in the following way: 10 g of fresh soil
137 was weighed and put into a 250 mL triangle bottle. Then we added 100 mL sterilized
138 0.85% NaCl solution, shaking for 30 min (speed at 170 $r \cdot min^{-1}$). The turbid liquid was
139 diluted with ultrapure water for 1000 times and incubated at 4℃ for 2-3 min. The
140 supernatant was taken into sterile culture dish and drew 150 μL per channel with the
141 eight channel pipetting gun into the EcoPlates. Finally the plates were cultured on 28℃
142 in biochemical incubator for 7 d. Absorbance at 590 and 750 nm was measured on
143 Biolog Microstation after 24, 48, 72, 96, 120, 144 and 168 of incubation hours. Optical
144 density (OD) value from each well was corrected by subtracting the control (blank well)
145 values from each plate well. The OD values obtained at 72 h represented the optima
146 range of optical density readings, so 72 h of incubation results was used for assessing
147 the microbial functional diversity and statistical analyses.

148 Statistical Analysis

149 In the process of Biolog data analysis, the AWCD value was calculated to reflect
150 the overall activity of soil microorganisms [31], and the Shannon-wiener index (H'),
151 McIntosh index (U) and Simpson index (D) at 72 h in the process of cultivation were
152 investigated to represent the metabolic functional diversity of soil microbial community

153 (Table 2)[32, 33].

154 **Table 2. Formulae for calculations**

Index	Definition	Formulae	Notes
Average well color development	/	$AWCD = \sum(C_i - R)/31$	C_i is the OD value of each substrate containing well (590-750 nm).
Simpson index	Measure of evenness	$D = 1 - \sum P_i^2$	R is the OD value of the black well.
Shannon-Wiener index	Measure of richness	$H' = -\sum P_i \ln P_i$	$P_i = (C_i - R)/\sum(C_i - R)$
McIntosh index	Measure of diversity	$U = \sqrt{\sum n_i^2}$	$n_i = (C_i - R)$

155 We performed an analysis of variance (ANOVA) using IBM SPSS 22.0 to explore
156 the significant effect of the amendments on AWCD [16], functional diversity indices
157 [33] and soil parameters, with the level of significance at $P < 0.05$. Fisher's Least
158 Significant Difference (LSD) test and Duncan test were used for the multiple
159 comparison. Moreover, Principle Component Analysis (PCA) was performed to
160 examine the similarities between different restoration patterns, and Redundancy
161 analysis (RDA) was to explore the soil environmental parameters that significantly
162 affecting the metabolic diversity of soil microbial communities. These analyses were
163 performed in Vegan packages in R 3.2.4 (http://www.r-project.org).

164 # Results

165 ## Soil characteristics

There was no significant difference in the SWC of the surface soil in dormant season between GL, FL, RL and PL patterns, while in growth season, the SWC of RL and PL were significantly higher than that of GL ($P < 0.05$) (Table 3). Overall, the SWC of GL was the lowest in the growing season, and FL obtained the highest value in the dormant season, which was about 4.6 times of the lowest. Soil pHs in GL and PL in dormant season were significantly lower than those in FL and RL, while in growing season, the pH was obviously higher in RL than in the other three patterns ($P < 0.05$). The contents of NH_4^+-N, NO_3^--N and DOC were the highest in PL in the dormant season, whereas in the growth season, the highest values of NH_4^+-N and NO_3^--N were in FL, and of DOC was in PL ($P < 0.05$).

The contents of SOC and TN were significantly higher in FL than in the other three patterns in both seasons ($P < 0.05$). The variation trend of SOC content during the dormant season was FL>PL>GL>RL, and that in growth season was FL>GL>PL>RL. For TN, the variation trend was FL>PL>GL>RL in both seasons. Furthermore, TP content did not change significantly in the dormant season, but showed significant differences in the growing season with the change trend of FL>PL>RL>GL. Generally among the four patterns, TP content in the growing season was higher than that in the dormant season. Besides, the variation range of TK content among the four patterns was 1.64-7.67 $g \cdot kg^{-1}$. Specifically, the TK contents were significantly higher in FL and PL than in GL and RL in the dormant season, while in the growing season, TK content in GL was significantly higher than those in RL and PL ($P < 0.05$).

Table 3. Effects of ecological restoration to soil physicochemical properties in

188 **different patterns**

Patterns	Seasons	SWC (%)	pH	SOC (g·kg^{-1})	DOC (mg·kg^{-1})	TN (g·kg^{-1})	NH_4^+-N (mg·kg^{-1})	NO_3^--N (mg·kg^{-1})	TP (g·kg^{-1})	TK (g·kg^{-1})
GL	DS	28.28 ± 0.73a	6.42 ± 0.10c	6.59 ± 1.09c	19.99 ± 7.55b	0.66 ± 0.10c	2.05 ± 0.41ab	0.35 ± 0.11bc	0.05 ± 0.00a	4.16 ± 0.49b
	GS	6.93 ± 0.80B	6.32 ± 0.03B	9.49 ± 0.29B	30.38 ± 4.05AB	0.82 ± 0.04B	0.81 ± 0.14B	0.05 ± 0.01B	0.22 ± 0.02C	6.63 ± 2.74A
FL	DS	31.69 ± 3.49a	7.14 ± 0.26b	23.92 ± 1.52a	35.68 ± 2.31ab	1.86 ± 0.09a	2.02 ± 0.25ab	0.60 ± 0.18ab	0.05 ± 0.02a	7.67 ± 0.20a
	GS	11.39 ± 0.95AB	6.21 ± 0.05B	23.81 ± 0.86A	32.31 ± 6.26AB	1.75 ± 0.05A	2.07 ± 0.14A	1.04 ± 0.26A	0.67 ± 0.04A	5.11 ± 0.37AB
RL	DS	27.38 ± 3.98a	8.27 ± 0.19a	5.06 ± 2.14c	22.98 ± 6.88ab	0.44 ± 0.12c	1.36 ± 0.13b	0.19 ± 0.01c	0.06 ± 0.01a	3.36 ± 0.74b
	GS	17.19 ± 2.90A	7.61 ± 0.14A	2.82 ± 0.48C	17.59 ± 2.78B	0.26 ± 0.02C	0.49 ± 0.11B	0.24 ± 0.16B	0.24 ± 0.03C	1.64 ± 0.52B
PL	DS	28.86 ± 0.90a	5.97 ± 0.14c	12.75 ± 0.28b	40.46 ± 4.83a	0.99 ± 0.06b	2.74 ± 0.30a	0.73 ± 0.06a	0.09 ± 0.03a	7.01 ± 0.36a
	GS	13.43 ± 1.56A	6.24 ± 0.30B	9.16 ± 0.66B	36.43 ± 6.17A	0.91 ± 0.05B	1.81 ± 0.48A	0.52 ± 0.07B	0.39 ± 0.01B	5.48 ± 0.24AB

189 Mean values ± Stand.Error (n=3).

190 Small letters in the same column indicate the significant difference between restoration patterns

191 in the dormant season (DS), and capital letters indicate that in growing season (GS) ($P < 0.05$).

192 Microbial activity and physiological diversity

193 Carbon substrate utilization, assessed via Biolog method, showed that different

194 restoration patterns modified the metabolic potential of the lakeside wetland soil

195 microbial community, and this effect varied in different seasons. Overall, the AWCD
196 values generally followed the same pattern with incubation time (leveling off after rapid
197 increase). As shown in Fig 1, AWCD values of the four patterns showed a rapid growth
198 trend at the initial stage of culture, with the fastest change rate at 72 h in both seasons.
199 Specially, the AWCD value of RL pattern was always the highest at the detection points
200 of each culture time, while the GL was the lowest.

201 **Fig 1. Average well color development (AWCD) of metabolized substrates in**
202 **Biolog EcoPlates based on 168 h incubation (n=3).**

203 A-Dormant season, B-Growing season.

204 The functional diversity indices of soil microbial community was calculated based
205 on AWCD values (Table 4). Results showed that in the dormant season, only the
206 McIntosh index (*U*) obviously differed within the four patterns, which indicated that
207 the *U* value in RL was significantly higher than that in GL, but not significantly differed
208 with FL or PL ($P < 0.05$). During the growing season, however, RL pattern had higher
209 Shannon-Wiener index (*H'*) compared to the other patterns ($P < 0.05$), and it ranked in
210 the following order: RL>FL>PL>GL. Besides, no significant difference on McIntosh
211 index and Simpson index was observed in this time.

212 **Table 4. Diversity indices based on the carbon-source utilization model for the**
213 **soil samples from GL, PL, RL and FL.**

Season	Pattern	McIntosh index (*U*)	Shannon-Wiener index (*H'*)	Simpson index (*D*)
Dormant	GL	2.78 ± 0.34B	2.87 ± 0.26A	0.93 ± 0.06A

season	PL	3.23 ± 1.52AB	2.95 ± 0.19A	0.94 ± 0.02A
	RL	3.81 ± 1.21A	3.02 ± 0.42A	0.95 ± 0.04A
	FL	3.12 ± 1.18AB	2.92 ± 0.13A	0.94 ± 0.03A
Growing season	GL	0.61 ± 0.27a	1.90 ± 0.13c	0.77 ± 0.04a
	PL	1.66 ± 0.43a	2.55 ± 0.07b	0.91 ± 0.00a
	RL	2.86 ± 1.13a	3.21 ± 0.14a	0.83 ± 0.12a
	FL	2.41 ± 0.47a	2.76 ± 0.06b	0.92 ± 0.01a

Mean values ± Stand.Error (n=3).

Capital letters indicate the significant difference between ecological restoration patterns of the same diversity index in the dormant season, and small letters indicate that in the growing season according to Duncan′s test ($P < 0.05$).

The EcoPlate carbon sources were divided into six biochemical categories, and the results from the 72-h incubation showed that in the dormant season, the relative utilization rate of carboxylic acids in RL was at a lower level, and that of amines was high. While the opposite trend was observed in GL, that is, carboxylic acids were most widely used, whereas the utilization of amines was less. Furthermore, soil microbial communities in FL and PL patterns had an average utilization efficiency for different carbon substrates (Fig 2A). During the growing season, the relative utilization rate of phenolic acids in GL was observed to be far more less than that in other three patterns, while the rate on carboxylic acids was significantly higher compared with RL, FL and PL (Fig 2B).

Fig 2. The relative utilization efficiency of carbon sources in soil microbial

community.

(a) CH-Carbohydrates, AA-Amino acids, CA-Carboxylic acids, PA-Phenolic acids, Pol-Polymers, Ami-Amines. (b) A-Dormant season, B-Growing season.

PCA analysis of metabolic characteristics of soil microbial communities

In order to determine the level of sites differentiation, the PCA ordination plots were performed though CLPPs. In the dormant season, the cumulative contribution rate of the first two axes was up to 77.49%, with PCA1 and PCA2 axes reaching 63.32% and 14.17%, respectively (Fig 3). Among the four ecological restoration patterns, GL and RL were distributed in a concentrated way, and the samples in GL, FL and PL grouped together which were distinct from samples in RL, indicating that the soil microbial communities in GL, FL and PL had similar carbon-source utilization patterns and were significantly different from those in RL.

Fig 3. PCA analysis of soil microbial carbon-source utilization in dormant season.

The dotted circles represent the 95% confidence interval.

The load values of 31 carbon sources on the two principal components were shown in Table 5. It can be seen that there were 4 types of carbon sources significantly correlated with PC1, and 10 types with PC2 ($P < 0.05$). Consequently, carbohydrates were the main carbon sources that distinguished the soil microbial metabolic characteristics from differential restoration patterns in dormant season.

250 **Table 5. Correlation coefficients of 31 carbon sources with PC1 and PC2 in**

251 **different seasons**

Category	Carbon sources (Code)	Dormant season		Growing season	
		PC1	PC2	PC1	PC2
Carbohydrates	β-Methyl-D-Glucoside (CH1)	0.6515	-0.0845	0.2163	**0.0066**
	D-Galactonic Acid-γ-Lactone (CH2)	0.2391	**-0.0042**	0.2572	-0.0522
	D-Xylose (CH3)	0.1023	**-0.0026**	**0.0213**	0.3812
	i-Erythritol (CH4)	-0.1165	0.1768	0.0974	**0.0172**
	D-Mannitol (CH5)	0.1555	0.3133	0.7302	-0.0844
	N-Acetyl-D-Glucosamine (CH6)	0.5176	**-0.0098**	0.3698	0.3988
	D-Cellobiose (CH7)	0.3415	0.1551	0.5898	-0.1076
	α-D-Glucose-1-Phosphate (CH8)	0.1052	**-0.0170**	0.3914	0.3516
	α-D-Lactose (CH9)	0.1495	**0.0263**	0.0977	0.0765
	D,L-α-Glycerol Phosphate (CH10)	**0.0113**	**0.0127**	0.0686	0.2336
Amino acids	L-Arginine (AA1)	0.4320	**0.0348**	0.4982	-0.2069
	L-Asparagine (AA2)	0.2791	0.1894	0.5797	-0.1466
	L-Phenylalanine (AA3)	-0.1487	**0.0207**	0.0779	0.3761
	L-Serine (AA4)	0.7437	-0.0739	0.3912	**0.0070**
	L-Threonine (AA5)	-0.1504	0.1903	0.3222	0.2502
	Glycyl-L-Glutamie Acid (AA6)	**-0.0174**	**0.0261**	**0.0138**	**0.0178**
Carboxylic acids	Pyruvic Acid Methyl Ester (CA1)	**0.0334**	0.2546	0.4467	-0.1693
	D-Galacturonic Acid (CA2)	-0.1068	0.1008	0.4313	**-0.0132**

	γ-Hydroxybuty ric Acid (CA3)	**0.0319**	0.2510	**0.0285**	0.1643
	D-Glucosaminic Acid (CA4)	-0.1013	0.1908	-0.0578	0.3297
	Itaconic Acid (CA5)	0.1867	0.0872	0.1654	**-0.0193**
	α-Ketobutyric Acid (CA6)	-0.1108	0.0829	**0.0381**	0.1511
	D-Malic Acid (CA7)	-0.6527	0.1955	0.1709	**0.0277**
Polymers	Tween 40 (Pol1)	0.1734	0.1440	0.2887	0.4086
	Tween 80 (Pol2)	0.1373	0.0731	0.3898	**0.0338**
	α-Cyclodextrin (Pol3)	0.1130	0.0731	**0.0268**	0.2874
	Glycogen (Pol4)	0.0906	**0.0028**	0.4082	-0.0541
Phenolic acids	2-Hydroxy Benzoic Acid (PA1)	-0.0548	0.0911	-0.1231	0.5939
	4-Hydroxy Benzoic Acid (PA1)	0.2847	0.1370	0.19329	-0.0766
Amines	Phenytethyl-amine (Ami1)	0.1725	0.1239	0.1489	**-0.0017**
	Putrescine (Ami1)	0.2075	0.1563	0.3163	-0.1461

Bold values indicates significant difference at the level of 0.05.

Considering the microbial communities in the growing season, PCA analysis showed that the contribution rate of PCA1 and PCA2 axes reached 52.19% and 26.27%, respectively (Fig 4). Among the four patterns, samples in GL and PL distributed in a concentrated way, whereas samples in FL and RL were relatively discrete and crossed each other. Besides, by calculating the loading values, we found 5 types of carbon sources highly correlating with PC1, and 9 types with PC2 ($P < 0.05$, Table 5). Combined with PC1 and PC2, carboxylic acids played a leading role in the metabolic characteristics of soil microbial communities among different restoration patterns in the growing season.

Fig 4. Principal component analysis of soil microbial carbon-source utilization in the growing season.

The dotted circles represent the 95% confidence interval.

RDA analysis on effect of soil environmental factors on microbial functional diversity

In the dormant season, the first two RDA axes explained 70.68% of the carbon-source utilization under the constraint ordination (Figure 5). The solid lines of soil pH and TK were shown to be longer than other parameters, which indicated their serious impacts on the microbial metabolism. Monte Carlo permutation tests further verified these significant effects of pH and TK, with correlation coefficient r^2 of 0.545 and 0.573, respectively ($P < 0.05$, Table 6). On the other hand, the dotted arrows showed that the carbon-source utilization in RL pattern were obviously different from PL, GL and FL that distributing in the positive axis of RDA1. It was consistent with the PCA results. The permutation tests further demonstrated the significant effects of different restoration patterns on functional diversity of soil microbial communities ($r^2 = 0.754$, $P < 0.01$, Table 6).

Furthermore, the plot also showed that various types of carbon-source utilized by microorganisms in different patterns were quite similar, and concentrated in the center of the axis (Fig 5). Based on the distribution distance between sites and carbon sources, we observed that the microbial communities in RL used more carbohydrates, GL sites preferred carboxylic acids, while PL and FL took the average utilization of different

283 carbon substrates because of the similar distance from most sources concentrating in
284 the center point.

285 **Fig 5. Redundancy analysis between the soil properties and microbial functional**
286 **diversity characteristics in dormant season.**

287 Carbon-source code is the same as in Table 5.

288 Based on the PCA and RDA analysis, the specific correlations between soil pH,
289 TK and 31 types of carbon-sources were tested through Pearson's correlation analysis
290 (Table A1). The results showed that in the dormancy season, there was a extremely
291 significant negative correlation between soil pH and L-Threonine (AA5, $r^2 = -0.714$, P
292 < 0.01), whereas soil TK had negative correlations with β-Methyl-D-Glucoside (CH1),
293 D-Galactonic Acid-γ-Lactone (CH2) and L-Arginine (AA1) ($P < 0.01$). Overall, there
294 were 6 in 10 kinds of carbohydrates and 5 in 6 of amino acids significantly correlated
295 with TK content ($P < 0.05$).

296

297 **Table 6. Monte Carlo permutation tests of the effect of environmental variables**
298 **on microbial carbon-source utilization in dormant and growing seasons**

Variables	Dormant season		Growing season	
	r^2	*P*	r^2	*P*
SWC	0.177	0.424	0.517	0.045*
pH	0.545	0.030*	0.028	0.878
SOC	0.071	0.723	0.319	0.190
TN	0.337	0.172	0.320	0.185

TP	0.345	0.162	0.208	0.348
DOC	0.240	0.317	0.057	0.738
NH_4^+-N	0.054	0.821	0.078	0.688
NO_3^--N	0.099	0.610	0.166	0.438
TK	0.573	0.017*	0.710	0.003**
Patterns	0.754	0.002**	0.615	0.01**

** indicates extremely significant correlation at the level of 0.01 (bilateral), and * indicates significant correlation at the level of 0.05 (bilateral).

In the growing season, the first two axes (RDA1 and RDA2) constructed the RDA plot, which could explain 48.85% and 19.86% of the total variation, respectively (Figure 6). The length of the solid arrow lines displayed that soil SWC and TK had significant effect on microbial carbon-source utilization, with r^2 calculated in permutation test of 0.517 and 0.710, respectively ($P < 0.05$). The dotted lines showed the obvious differentiation between GL, PL and RL, whereas there were similarities of the metabolic diversity in RL and FL. Moreover, the distribution of the carbon-sources in RDA ordination plot displayed that the substrates utilized by microorganisms in different patterns were relatively discrete, and the sources that may be used by certain communities were largely scattered around the positive half of the RDA1 axis (Fig 6). Overall, amino acids, polymers and phenolic acids were increasingly utilized in RL, PL and FL patterns, which were different from the metabolic characteristics in GL.

Pearson's correlation analysis indicated that in the growing season, SWC positively affected the utilization of several carbon sources, especially for i-Erythritol (CH4), D-

Mannitol (CH5), N-Acetyl-D-Glucosamine (CH6) and D-Cellobiose (CH7) in carbohydrates ($P < 0.01$), and Pyruvic Acid Methyl Ester (CA1), Itaconic Acid (CA5) and D-Malic Acid (CA7) in carboxylic acids ($P < 0.05$). However, the correlations between soil TK and carbon sources were just the opposite, for the significant negative relations with i-Erythritol (CH4), D-Mannitol (CH5), N-Acetyl-D-Glucosamine (CH6), D-Cellobiose (CH7) and α-D-Glucose-1-Phosphate (CH8) in carbohydrates ($P < 0.01$), and D-Galacturonic Acid (CA2), Itaconic Acid (CA5) and D-Malic Acid (CA7) in carboxylic acids ($P < 0.05$) (Table in S1 Table).

Fig 6. Redundancy analysis (RDA) between the soil properties and microbial functional diversity characteristics in growing season.

Carbon-source code is the same as in Table 5.

Discussion

In the present study, the Biolog EcoPlates™ method was used to monitor the soil microbial functional diversity of Chaohu lakeside wetland. The AWCD values recorded the overall ability of microbial communities to utilize 31 single carbon-sources within 168 h [34], showing that the carbon-source utilization rate increased significantly after 24 hours of incubation, and their utilization capacity was obviously different among four patterns. Overall the microbial metabolic activity was lower in GL pattern than in other three patterns, and the relatively lower diversity index also demonstrated the poorer microbial populations in GL. As the derelict land around the lake, GL sites are covered by only weed, without any trees and shrubs due to the heavy clay and hardening. Previous research has reported that the abandoned fields are often characterized by less

337 diverse plant communities [35, 36]. Besides, soil C and N fractions are derived from
338 the decomposition of litter [37], the release of root exudates, and rhizodeposition [38].
339 Lack of aboveground cover could decrease the quantity and quality of litter and roots,
340 thus lowering the soil nutrient content and cycling, which may be negative for the soil
341 microbial diversity.

342 In our study, soil microorganisms were observed to have various preferences on
343 the carbon-source utilization in different seasons, especially for carbohydrates in
344 dormant season and carboxylic acids in growing season. Carbohydrates, which belong
345 to the soil active organic carbon pool [39], provide energy and substrates for
346 microorganisms, and are significantly related to microbial activity [40, 41]. Thereby
347 many aerobic and facultative heterotrophs choose carbohydrates as their main sources
348 to realize their functions, the process of which involves the oxidation of simple or
349 complex carbohydrates [42-45]. Moreover, carboxylic acids are the important part of
350 the organic acid that can affect the release of heavy metal ions in soil and improve their
351 activities [46]. Lin et al. [47] found that the increase of heavy metals and their
352 availability in soil enhanced the metabolic utilization of carboxylic acids. In summer,
353 water eutrophication may lead to more nutrients enriching into the wetland soil, which
354 may stimulate the microorganisms to use more carboxylic acids to adsorb and degrade
355 heavy metals. This is consistent with Li et al. [48] who reported that carboxylic acids
356 and carbohydrates were the sensitive carbon-sources affecting the metabolic function
357 of microbial communities in the Songjiang wetland under the effect of four kinds of
358 disturbances.

359 Interestingly, when taking the environmental factors into consideration, the
360 situation of functional metabolism became much more complicated in various
361 restoration patterns from RDA results. In dormant season, the carbon sources like
362 carbohydrates and carboxylic acids were used widely, and the microbial communities
363 in four patterns showed a similar way in selecting substrates. However, the microbial
364 communities showed more versatile substrate utilization patterns in growing season
365 than in dormant season. For example, RL increased the substrate utilization of amino
366 acids, whereas microorganisms in PL and FL used more phenolic acids and polymers.
367 Consequently, not only the easily degraded compounds but also the complex ones were
368 consumed to satisfy the metabolic requirement of soil microorganisms. Adam et al. [12]
369 also reported that in spring and summer, the frequency of nitrogen-rich carbon sources
370 (amino acids) [45] used by microbial communities was higher than that of
371 carbohydrates and carboxylic acids. In addition to utilizing carbon, these microbial
372 communities also use amino acids as nitrogen source and combine with ammonia side
373 chains. Ammonium is then made into organic molecules, such as amino acids and
374 proteins [49]. Moreover, phenolic acids are a major class of phenolic compounds made
375 by plants [50], and denaturing gradient gel electrophoresis (DGGE) results obtained by
376 Zhou and Wu [51, 52] have proved that the phenolic acid supplementations can
377 significantly alter the soil bacterial community composition. Besides, polymers are
378 complex carbon substrates, and previous research has shown that when the growth of
379 soil bacteria was inhibited due to lack of required nutrients (e.g., nitrogen or phosphate)
380 in the presence of excess carbon, the glycogen may accumulate in bacteria [53]. In our

study, the high use of these groups (amino acids, phenolic acids and polymers) in RL, PL and FL patterns might be related to the availability of various substrates in growing season. After vegetation restoration, soil microbial populations and diversity increased significantly compared to the shoaly abandoned land, which stimulate their competition for utilizing different kinds of substrate, thus enhance the complex carbon-source degradation under the suitable hydrothermal condition to satisfy their multiple functional needs.

Furthermore, CLPPs seem to be specific for land use, soil management and soil texture [13]. Our research showed that soil TK contents was the dominant factor affecting the microbial functional diversity in both dormant and growing seasons. After nitrogen and phosphorus, potassium (K) is one of the major nutrients required by vegetation, and it plays a major role in the activation of several metabolic processes including protein synthesis, photosynthesis, enzyme activation, etc [54]. Specially, K acts as a key to activate enzymes to metabolize carbohydrates for the synthesis of amino acids and proteins [55], and previous studies reported diverse groups of soil microorganisms took an active part in solubilizing the fixed forms of K into available forms which plants are easy to absorb [56, 57]. This may explain the significant relationship between TK and substrates of carbohydrates and amino acids used by soil microbial communities observed in our study. The utilization ability of communities to different carbon-sources, however, depends to a large extent on the types and inherent properties of microorganisms, as Barra Caracciolo et al. [58] observed the shifts of soil microbial composition determined their functional diversity, and finally had different

403 impacts on the ecosystem processes. The EcoPlates™ method can be used to describe
404 the utilization of microbial communities to a single type of carbon-sources, whereas the
405 complex edaphic environment could not be simulated. Therefore, future research on
406 microbial compositional and structural information is needed to help to understand the
407 mechanism about the metabolic shifts of microbial communities during the ecological
408 restoration in Chaohu lakeside wetland.

409 Conclusions

410 This study showed that Chaohu lakeside wetland soils with different
411 biogeochemical properties had microbial communities that exhibit distinct catabolic
412 responses to a range of carbon-sources. The AWCD values and diversity indices
413 indicated soils in RL, PL and FL patterns had the higher overall metabolic activity of
414 different substrates, whereas the microbial communities in GL had the relative lower
415 ability. Seasonal variation of functional diversity was observed between dormant and
416 growing seasons, and the CLPPs results clearly distinguished the metabolic characters
417 in natural and artificial restoration patterns from that in the abandoned shoaly land by
418 their capacity to utilize more complex carbon sources, such as amino acids, phenolic
419 acids and polymers, which may be linked to differences in the soil microbial
420 composition, vegetation types, soil physicochemical properties and hydrothermal
421 condition, etc. All the soil parameters and Biolog data demonstrate the positive effect
422 of ecological restoration on increasing the microbial activity and functional diversity.
423 This study also displayed a close linkage between physicochemical properties (TK, pH
424 and SWC) of lakeside wetland soil and the associated microbial functional activities,

425 which may provide basic information regarding the environmental protection and
426 management of Chaohu wetland.

427

428 Author Contributions

429 Conceptualization: ZT.

430 Data curation: ZT.

431 Formal analysis: WF HW.

432 Investigation: HW XC.

433 Methodology: ZT WF.

434 Supervision: XX.

435 Writing – original draft: ZT.

436 Writing – review & editing: XX.

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Data Availability Statement

All relevant data are within the paper and its Supporting Information files.

Conflicts of Interest

The authors have declared that no competing interests exist.

Supporting information

S1 Table. Correlation coefficients of the microbial carbon-source utilization with the soil environmental parameters in dormant and growing seasons. ** indicates extremely significant correlation at the level of 0.01 (bilateral), and * indicates significant correlation at the level of 0.05 (bilateral). The carbon-source code is the same as in Table 3.

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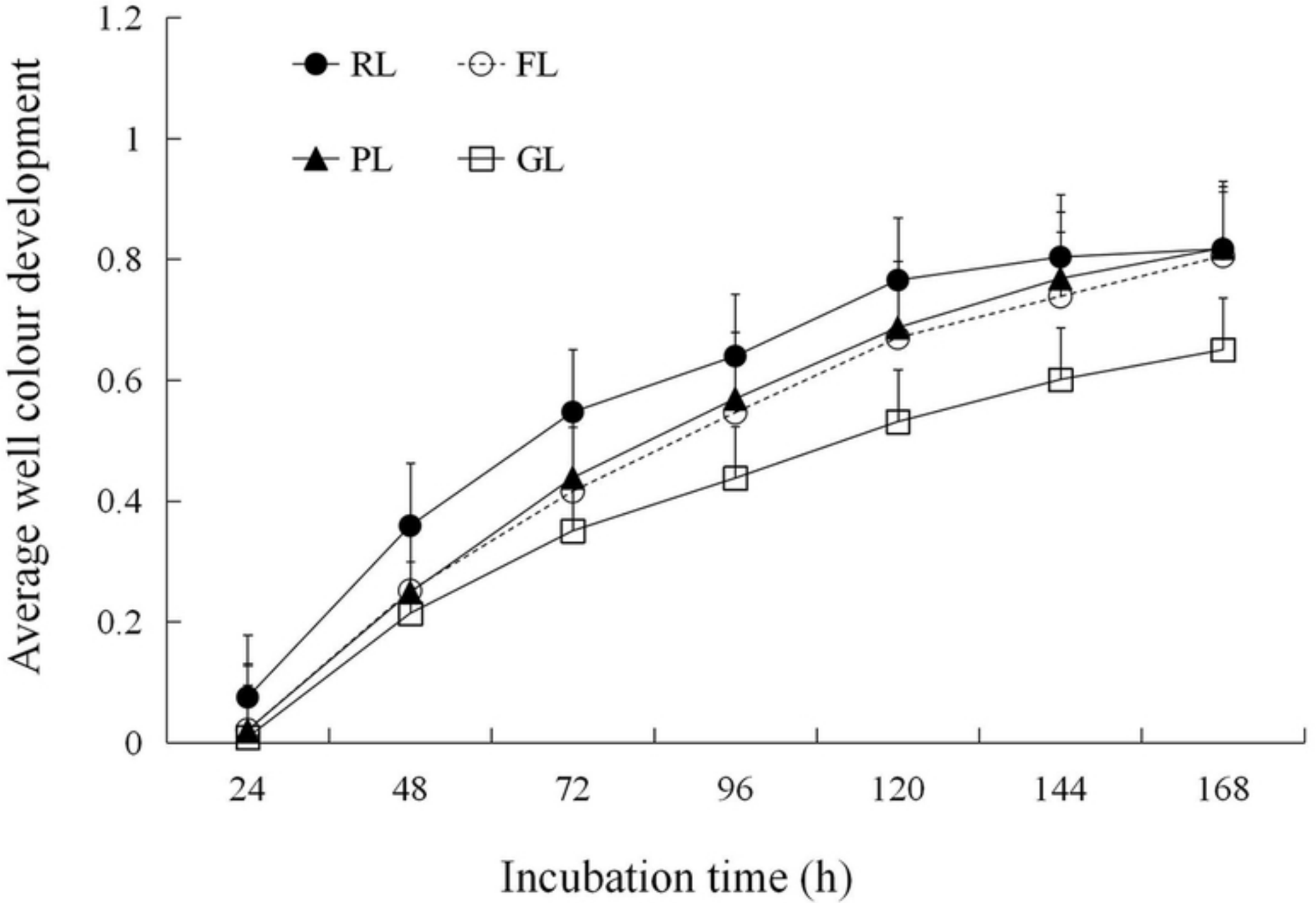
621

A


RL
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PL
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Average well colour development
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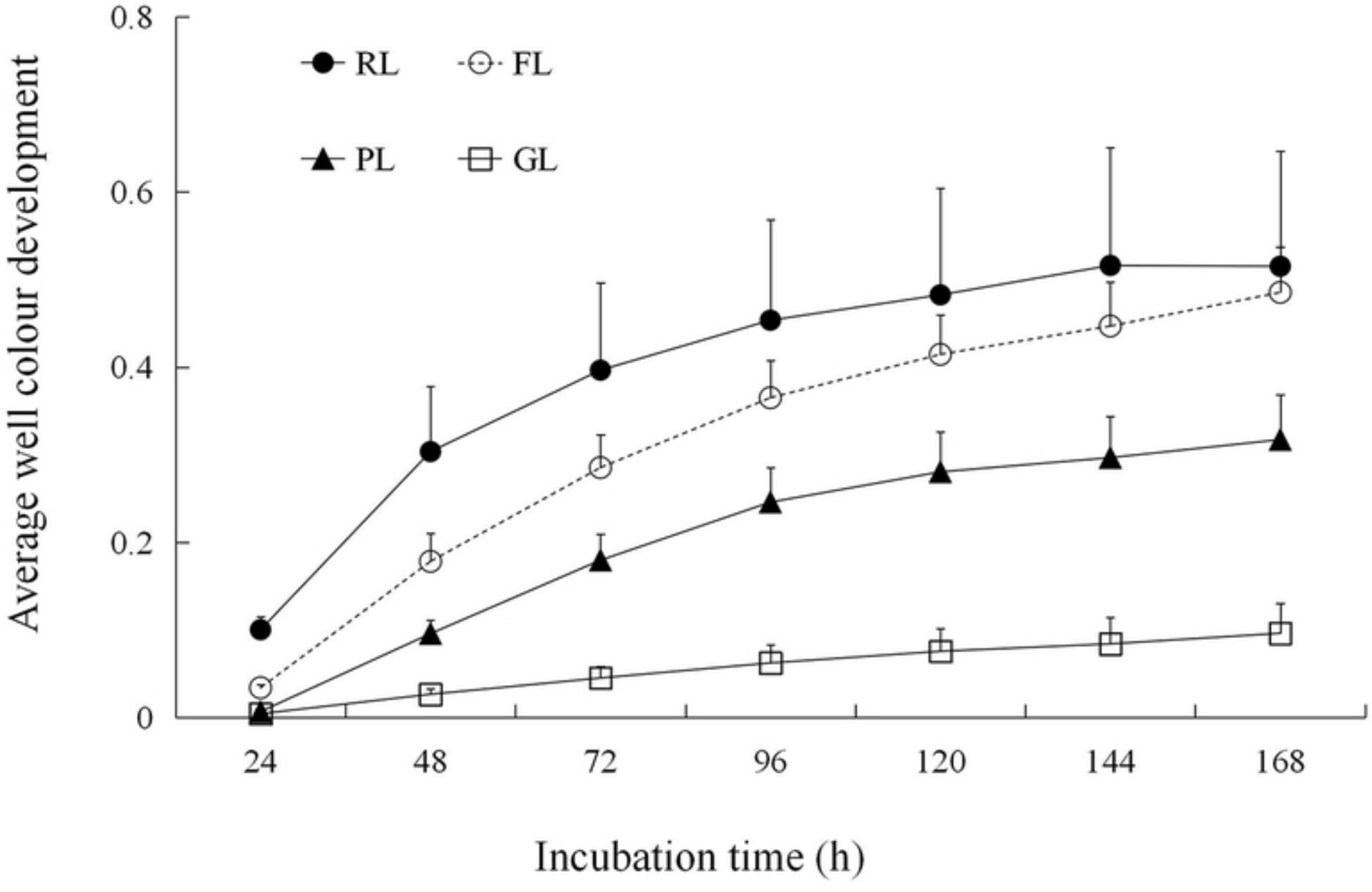
Incubation time (h)

Fig 1A

B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Incubation time (h)

Fig 1B

A

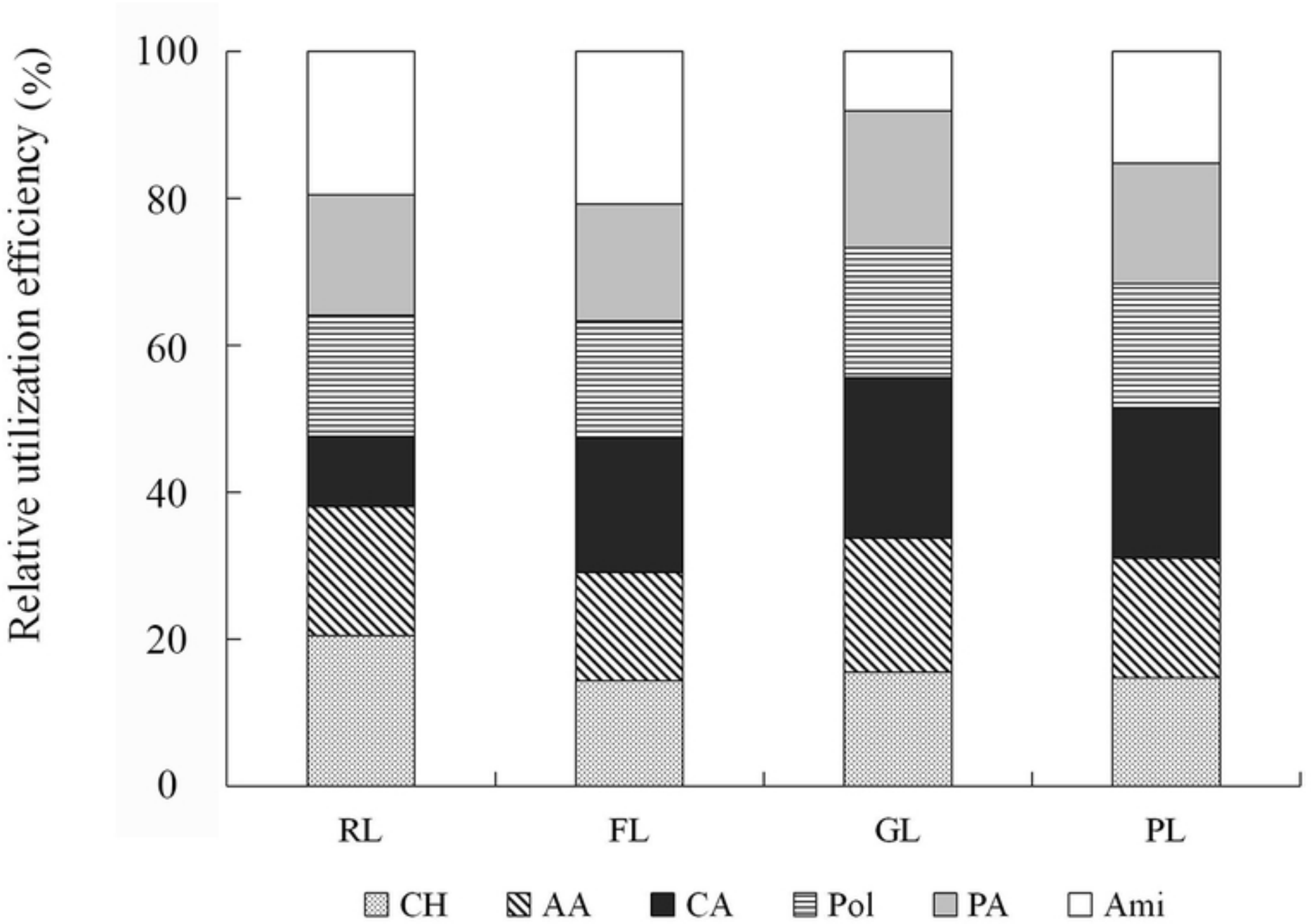
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Fig 2A

B

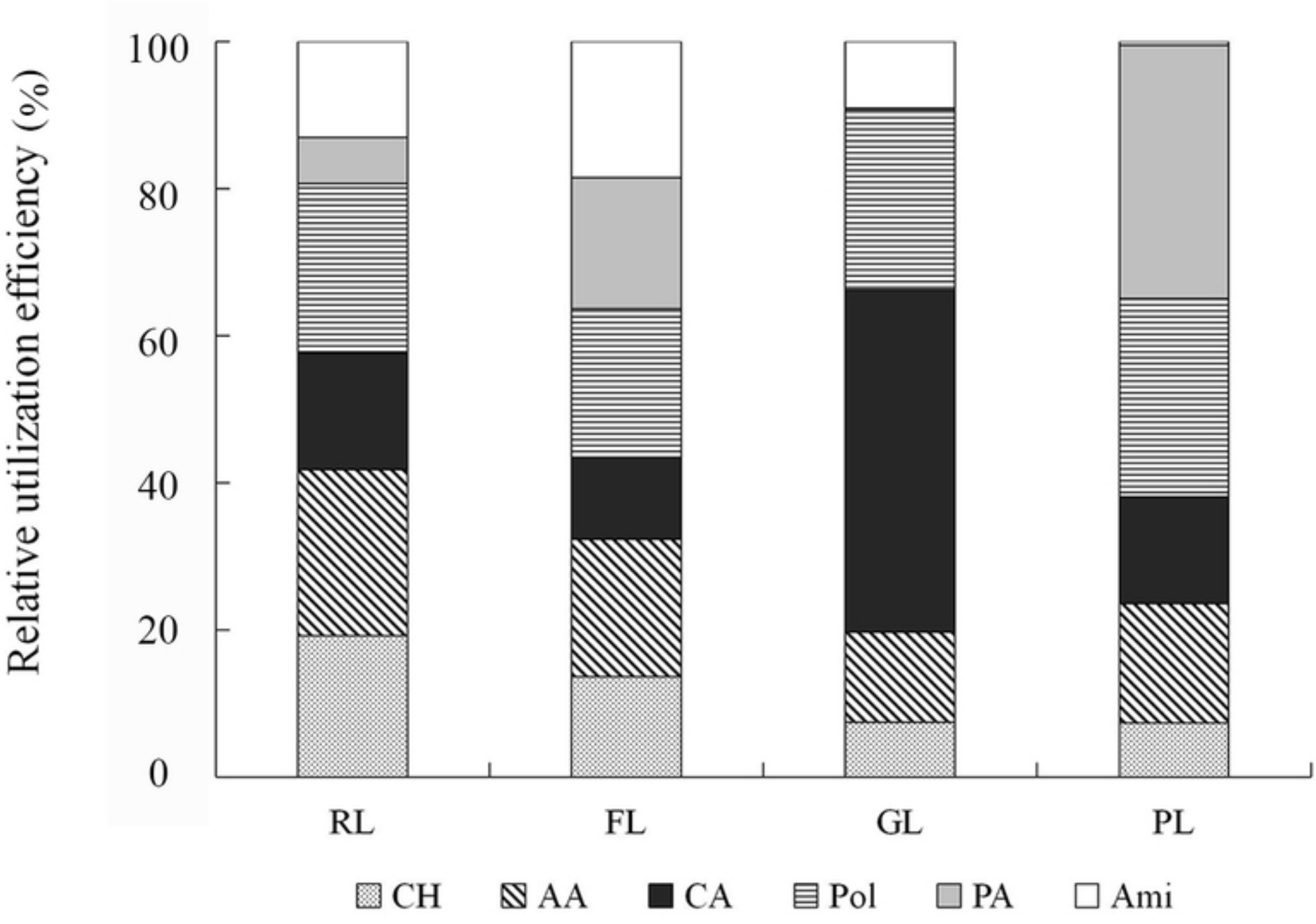
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Fig 2B

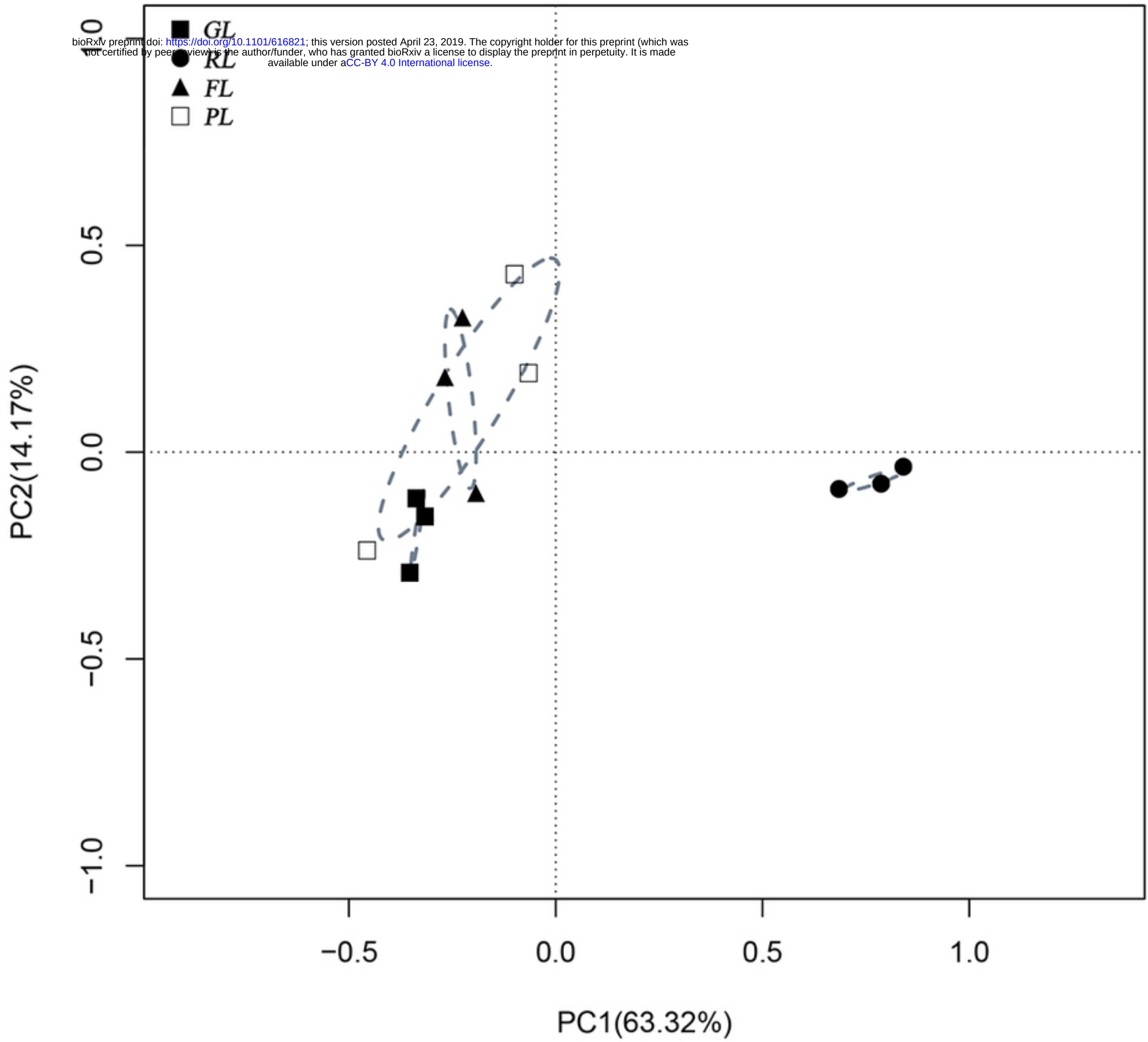

Fig 3

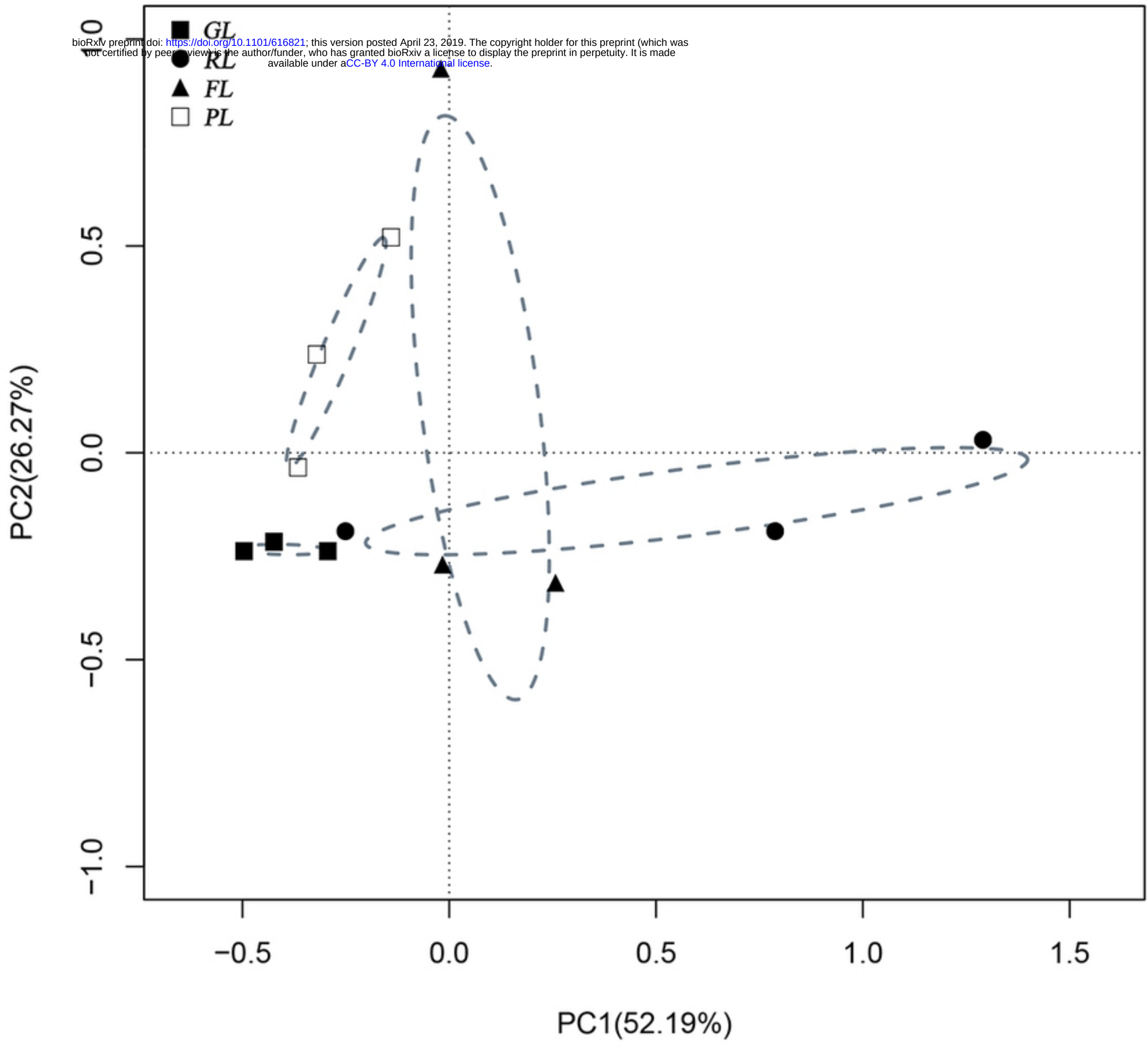

Fig 4

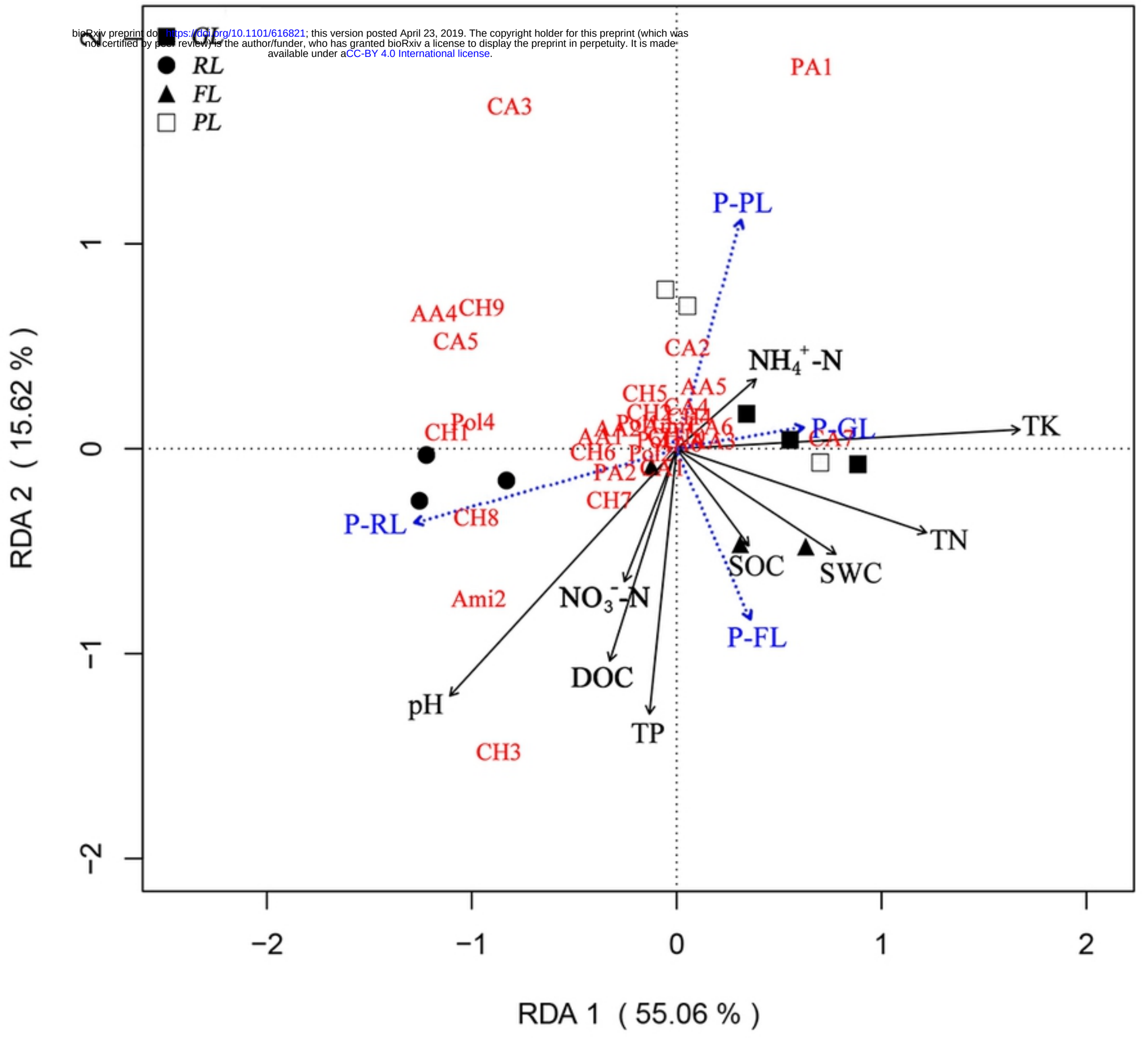

Fig 5

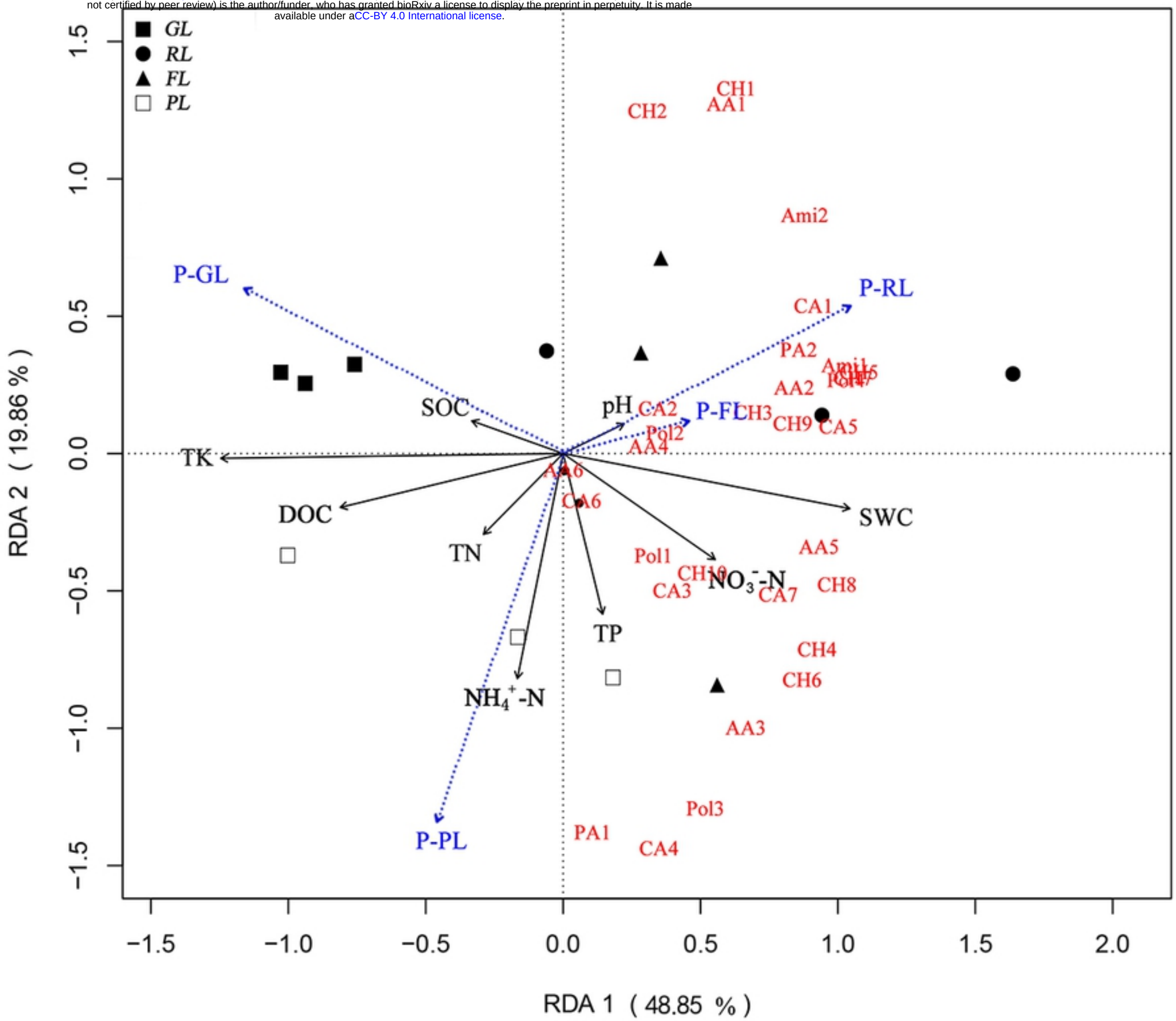

Fig 6