agronomy

Supplementary Information

Phospholipid fatty acids method (PLFA)

PLFA method detection is divided into four steps: (1) Extraction: 8.0 g of freeze-dried soil sample (−70 °C) was weighed in a triangular flask, then 15 mL of Bligh-Dyer extract (chloroform, methanol, citric acid buffer in the volume ratio of 1:2:0.8 *v*/*v*/*v*) was added, sonicated for 30 min in a dark environment, shaken for 30 min (200 rpm), centrifuged for 10 min (4000 rpm) and then extracted again. Transferred the supernatant from the previous step to a 50 mL glass tube wrapped with tinfoil, added 10 mL of BD extract, shook for 30 min in the dark, centrifuged for 10 min (4000 rpm), and transferred the supernatant to a 50 mL tube; added 4 mL of phosphate buffer and 4 mL of chloroform to the tube, vortexed and shook until the solution became milky white. The tube was sealed with tin foil and placed in a refrigerator at 4 °C in a dark environment overnight. The lower layer was pipetted out into a 10 mL screw-top tube and blown dry with nitrogen. (2) Purification: The silica gel column was fixed on the extraction rack, washed with 2 mL of chloroform, and left to dry after the washing solution was completely filtered. The sample was redissolved by adding 2 mL of chloroform and 1 mL of chloroform, and 4 mL of chloroform (2 mL each time, full) was added twice to elute neutral lipids, 12 mL of acetone (2 mL each time, full) was added six times to elute glycolipids, and 8 mL of methanol (2 mL each time) was added four times to elute phospholipids, after which the methanol was blown dry with nitrogen. The product was stored frozen at −20 °C. (3) Methylation: 1 mL of toluene and methanol in a volume ratio of 1:1 and 1 mL of 0.2 M KOH was added to the bottom of the tube (sample); the solution was vortexed and mixed well and then water-bath heated for 30 min; after that, 1 M acetic acid solution was added to adjust the pH to neutral (about 7); 5 mL of hexane and chloroform in a volume ratio of 4:1 was added into the solution (if turbidity appears, quantitative NaCl needed to be added). Then the solution added 3 mL of ultrapure water was vortexed for 5–30 s and centrifuged for 5 min (2000 rpm). The supernatant was aspirated into a GC bottle, blown with nitrogen until dry and stored under dark conditions at −20 °C. (4) Measure: The sample to be measured in the GC bottle was dissolved with 2 × 100 μL of hexane (vortex), transferred to the interpolation tube, and the phospholipid fatty acid profile was measured on the machine (GC-MS, Agilent Technologies, Palo Alto, CA, USA). Based on the differences in the properties of phospholipid fatty acids, the different types and contents of PLFAs in the phospholipid fatty acid profiles were used to identify the bacterial abundance and fungal abundance, as well as the total microbial biomass. (Chen et al., 2018; Frotegard et al., 2011; Chen et al., 2015; Swallow et al., 2009; Wan et al., 2015)

References

[1] Chen, H.M.; Ma, J.Y.; Wei, J.X.; Gong, X.; Yu, X.C.; Guo, H.; Zhao, Y.W. Biochar increases plant growth and alters microbial communities via regulating the moisture and temperature of green roof substrates. Sci. Total Environ. 2018, 635, 333–342.

[2] Frostegård, A.; Bååth, E. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils. 1996, 22, 59–65.

[3] Chen, D.; Lan, Z.; Hu, S.; Bai, Y. Effects of nitrogen enrichment on belowground communities in grassland: Relative role of soil nitrogen availability vs. soil acidification. Soil Biol. Biochem. 2015, 89, 99–108.

[4] Swallow, M.; Quideau, S.A.; MacKenzie, M.D.; Kishchuk, B.E.; Microbial community structure and function: the effect of silvicultural burning and topographic variability in northern Alberta. Soil Biol. Biochem. 2009. 41 (4):770–777.

[5] Wan, X.H.; Huang, Z.Q.; He, Z.M.; Yu, Z.P.; Wang, M.H.; Davis, M.R.; Yang,Y.S. Soil C:N ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations. Plant Soil. 2015. 387 (1–2):103–116. https://doi.org/10.1007/s11104-014-2277-4.

Description of phosphorus source interference in culture medium

Supplementation experiment (dissolved phosphate, Figure 1). The bacterial liquid was filtered through 0.2 μm polycarbonate membrane filters and the filtrate was analysed for soluble reactive phosphate (PO_4) using molybdenum blue method (Grasshoff et al., 1999). We found only 0.171 mg/L of phosphate remained in the medium after 5 days. In contrast to our experiment, which used only 3 mL of medium, only 0.000513 mg of phosphate in total. Meanwhile, combined with previous studies (Li and Dittrich, 2018), it was confirmed that the phosphate in the medium is almost depleted before the end of the microorganism logarithmic phase, so this indicator has a negligible effect on the formation of heavy metal phosphate in our test. Our previous study (Chen et al., 2019, 2020) found that PSB could release ~45 mg/L soluble total phosphorus in the LB medium after 3 days. Even so, the total P content in the medium (3 mL) added in our experiment was 0.135 mg, which had little effect on the formation of heavy metal phosphate.

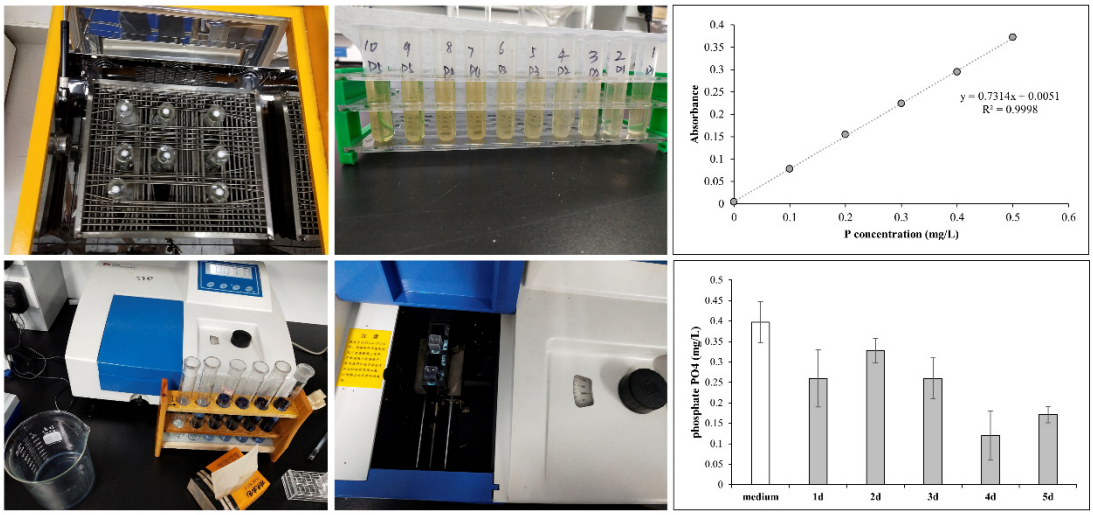

Figure S1. The soluble reactive phosphate (PO_4) of bacterial liquid and medium during five-day culture.

Table S1. Test results of normal distribution of index data. (In order to unify data comparability, all data are normalized and mean processed. According to the analysis results, most of the data meet the requirements of normality (p value > 0.05). Due to the low number of samples in this test, it is considered that p value > 0.01 is also referential.)

	Normality test					
	Kolmogorov-Smirnov [a]			Shapiro-Wilk		
	Statistics	df	Significance (p value)	Statistics	df	Significance (p value)
pH after 30 days	0.199	15	0.112	0.866	15	0.029
Water loss	0.152	15	0.200 *	0.879	15	0.046
Moisture content	0.193	15	0.138	0.895	15	0.079
TC	0.232	15	0.029	0.907	15	0.123
TN	0.382	15	0.000	0.676	15	0.000
AP	0.271	15	0.004	0.765	15	0.001
AK	0.335	15	0.000	0.728	15	0.000
water soluble fraction of Pb	0.174	15	0.200 *	0.905	15	0.113
exchangeable fraction of Pb	0.159	15	0.200 *	0.882	15	0.051
acid soluble fraction of Pb	0.221	15	0.047	0.839	15	0.012
non-bioavailable fraction of Pb	0.194	15	0.135	0.864	15	0.028
water soluble fraction of Cd	0.298	15	0.001	0.754	15	0.001
exchangeable fraction of Cd	0.204	15	0.095	0.834	15	0.010
acid soluble fraction of Cd	0.281	15	0.002	0.811	15	0.005
non-bioavailable fraction of Cd	0.202	15	0.101	0.893	15	0.076
bacterial biomass	0.176	15	0.200 *	0.925	15	0.229
fungal biomass	0.110	15	0.200 *	0.969	15	0.842
total microbial biomass	0.186	15	0.171	0.894	15	0.078

*. Lower limit of true significance.
[a]. Lilliefors Significant level correction.

Table S2. Parameters in Act2 program module of the GWB software.

Diagram species	**Pb^{2+}**	**500.00 mg L^{-1}**	**Cd^{2+}**	**271.24 mg L^{-1}**
X axes	H^+	from to	pH =5 pH =9	Increment: 1
Y axes	$H_2PO_4^-$	from to	Log a = 0 Log a = 120	Increment: 1
In the presence of	H_2O	1	Temperature	25.0 °C
	SO_4^{2-}	10.00 mg L^{-1}		
	Ca^{2+}	0.00 mol L^{-1}		
	F^-	0.00 mol L^{-1}		
	CO_2	−3.50 mol L^{-1}		
	Cl^-	50.00 mg kg^{-1}		

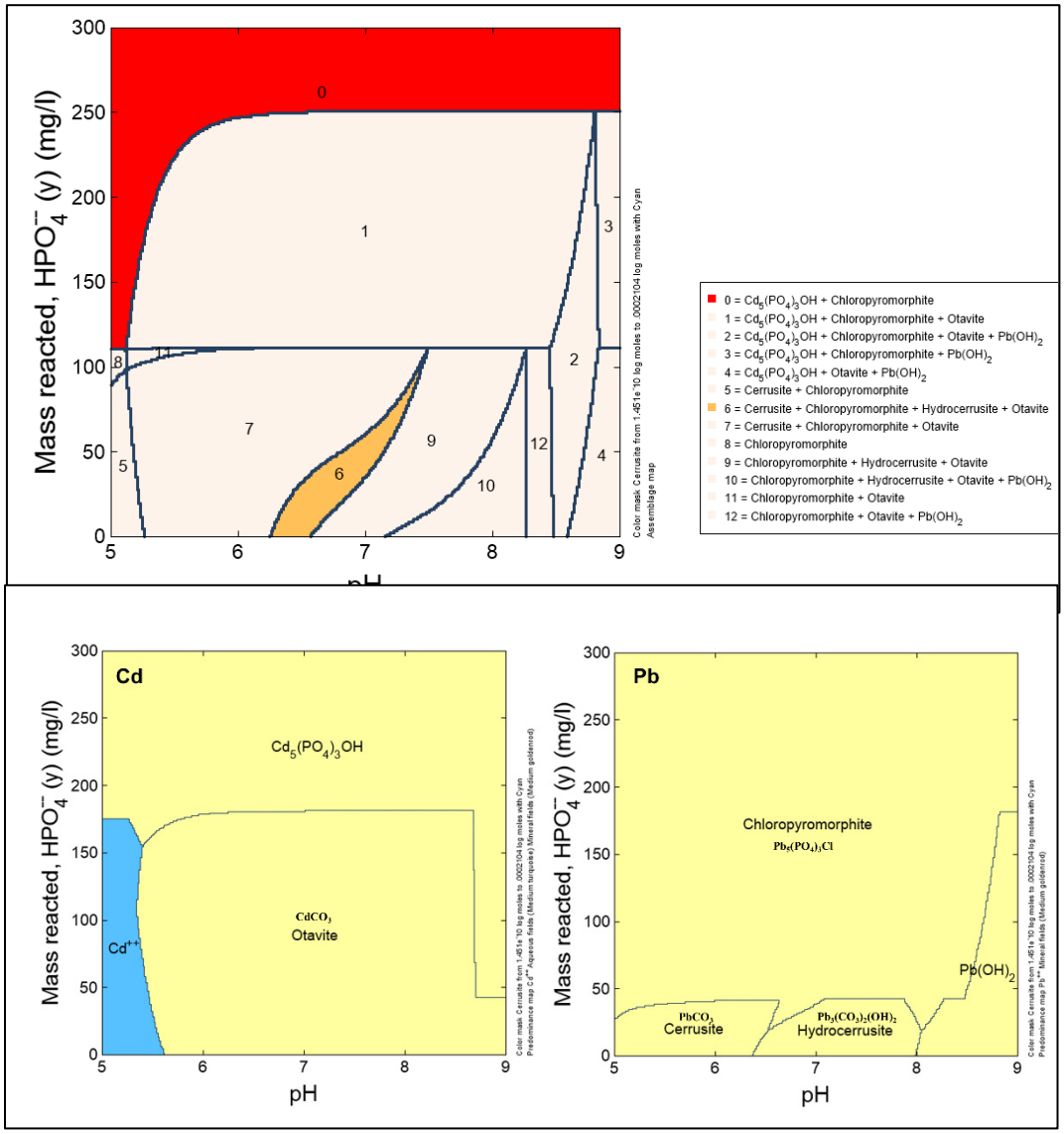

Figure S2. Final minerals (Pb + Cd) formed at high P concentration in the GWB model.