1   **Supplementary material**

2   **Stronger microbial nutrient limitations in subsoil along the precipitation gradient of**

3   **agroecosystem: Insights from soil enzyme activity and stoichiometry**

5   Jingjing Yang [a,b], Pingting Guan [a], Peng Zhang [b], Yunga Wu [a,b], Deli Wang [c], Donghui Wu

6   [a,b,d,e]*

7   [a] *State Environmental Protection Key Laboratory of Wetland Ecology and Vegetation Restoration, School*

8   *of Environment, Northeast Normal University, Changchun 130117, China*

9   [b] *Key Laboratory of Wetland Ecology and Environment, Institute of Northeast Geography and Agroecology,*

10   *Chinese Academy of Sciences, Changchun 130102, China*

11   [c] *Institute of Grassland Science, Key Laboratory of Vegetation Ecology of the Ministry of Education, Jilin*

12   *Songnen Grassland Ecosystem National Observation and Research Station, Northeast Normal University,*

13   *Changchun 130024, China*

14   [d] *Key Laboratory of Vegetation Ecology, Ministry of Education, Northeast Normal University, Changchun*

15   *130024, China*

16   [e] *Jilin Provincial Key Laboratory of Animal Resource Conservation and Utilization, Northeast Normal*

17   *University, Changchun 130117, China*

18

19   * Corresponding author.

20   School of Environment, Northeast Normal University, No. 2555, Jingyue Street, Changchun

21   130117, Jilin Province, China

22   E-mail address: wudonghui@neigae.ac.cn

23   **Table S1**

24   Site detail across a gradient of precipitation region.

25   **Table S2**

26   Relationships between the angle and length of vector and precipitation among soil profiles.

27   Dependent variables are vector angle and length, depth is a grouping factor with five levels,

28   and the precipitation are covariates in ANCOVA models. A significant depth × covariate

29   interaction would indicate that the slope of the vector–precipitation relationship differs

30   among soil profiles. Depth followed by a different letter are significantly different

31   (Bonferroni, $P < 0.05$).

32   **Figure S1.** A schematic diagram of enzyme methodology. Blank: 250 µl acetate buffer.

33   Standard: 50 µl of standard substrate and 200 µl acetate buffer. Substrate: 50 µl of 200 µM

34   enzyme substrate solution in 200 µl of acetate buffer. Sample: 50 µl of acetate buffer and 200

35   µl of soil sample suspension. Quench Control: 50 µl of the standard substrate (10 µM 4-

36   methylumvelliferyl or 7-amino-4-methylcoumarin) plus 200 µl of soil sample suspension.

37   Assay: 50 µl of 200 µM enzyme substrate solution and 200 µl of soil sample suspension.

38

Table S1

Site detail across a gradient of precipitation region.

Site	Location	Altitude (m)	MAT (°C)	MAP (mm)	Soil type
Changling (CL)	44°35'N, 123°30'E	140	6.2	381	Salt-alkali
Sijianfang (SJF)	44°18'N, 124°07'E	190	6.2	416	Light chernozem
Nongan (NOA)	44°11'N, 124°37'E	250	5.5	493	Chernozem
Changchun (CC)	43°54'N, 125°13'E	232	6.3	586	Black soil
Dongliao (DL)	42°54'N, 125°25'E	310	5.9	688	Dark brown soil
Longwan (LW)	42°22'N, 126°26'E	670	5.9	756	Dark brown soil
Baihe (BH)	42°23'N, 127°05'E	780	4.1	840	Dark brown soil

Table S2

Relationships between the angle and length of vector and precipitation among soil profiles.

Dependent variables are vector angle and length, depth is a grouping factor with five levels,

and the precipitation are covariates in ANCOVA models. A significant depth × covariate

interaction would indicate that the slope of the vector–precipitation relationship differs

among soil profiles. Depth followed by a different letter are significantly different

(Bonferroni, $P < 0.05$).

Dependent variable and covariate	Depth (cm)				
	0-10	10-20	20-30	30-40	40-50
vector angle × precipitation	a	a	b	b	b
vector length× precipitation			a	a	a
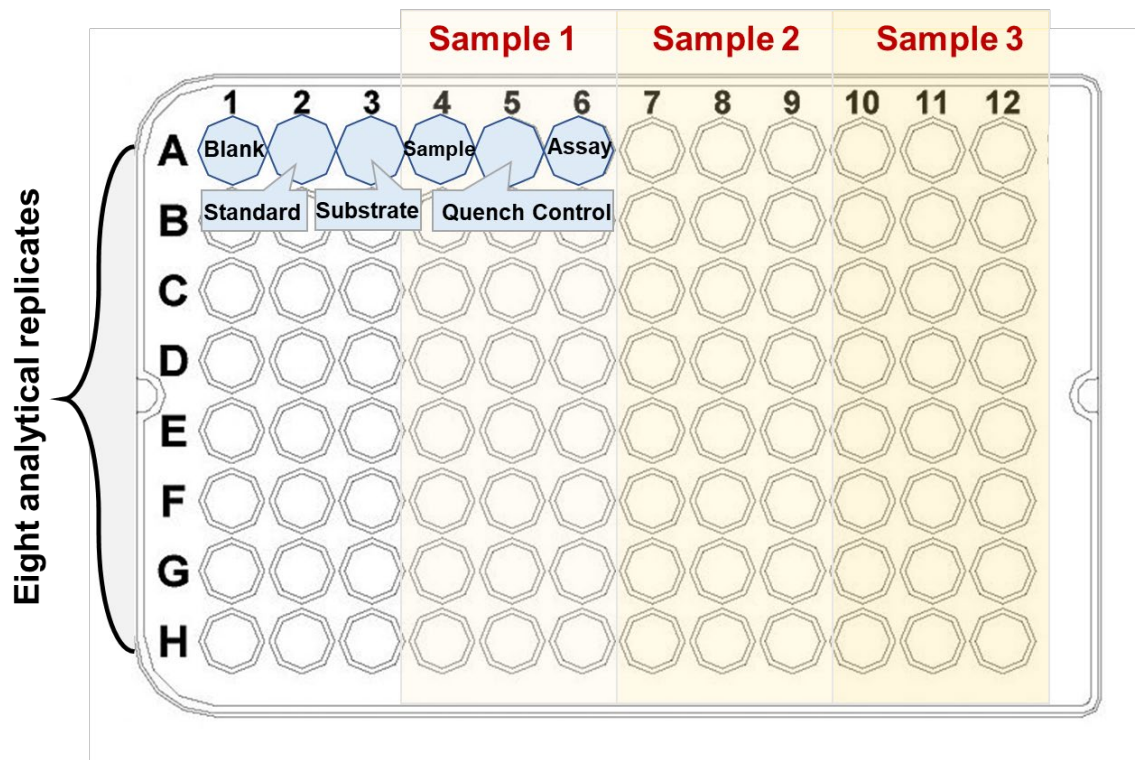
Figure S1. A schematic diagram of enzyme methodology. Blank: 250 μl acetate buffer.

Standard: 50 μl of standard substrate and 200 μl acetate buffer. Substrate: 50 μl of 200 μM

enzyme substrate solution in 200 μl of acetate buffer. Sample: 50 μl of acetate buffer and 200

μl of soil sample suspension. Quench Control: 50 μl of the standard substrate (10 μM 4-

methylumvelliferyl or 7-amino-4-methylcoumarin) plus 200 μl of soil sample suspension.

Assay: 50 μl of 200 μM enzyme substrate solution and 200 μl of soil sample suspension.