Chen Guowei (Orcid ID: 0000-0002-3093-9866)

Wang Gang (Orcid ID: 0000-0002-3201-6950)



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# **Title:** Phage-bacterium interactions and nutrient availability can shape C and N retention in microbial biomass

**Running title:** C and N retention in microbial necromass associated with phage lysis

Hanqing Wu[a, 1], Sichen Wan[a, 1], Chujin Ruan[a, b], Xinyao Niu[a], Guowei Chen[c], Ying Liu[a], Kun Zhu[a], Rainer Schulin[d], Gang Wang[a, *]

[a] *Department of Soil and Water Sciences, China Agricultural University, Beijing* 100193*, China*

[b] *Swiss Federal Institute of Aquatic Science and Technology, Dübendorf* 8600*, Switzerland*

[c] *College of Civil Engineering, Hefei University of Technology, Hefei* 230009*, China*

[d] *Department of Environmental System Science, ETH Zurich, Zurich* 8092*, Switzerland*

*** Corresponding Author:** Prof. Dr. Gang Wang, Department of Soil and Water Sciences, China Agricultural University, Beijing 100193, China.

Tel.: +86-10-6273558 E-mail address: gangwang@cau.edu.cn (Gang Wang)

[1] These authors contributed equally to this work.

**Abstract**

Phage-bacterium interactions influence soil microbial life and ecological functions, including microbial evolution, community patterns, and nutrient cycling. However, understanding of phage-mediated soil bacterial lysis dynamics, and impacts on soil carbon (C) and nitrogen (N) cycling remains elusive. Here, we employed a short-term laboratory incubation microcosm model system (which did not contain actual soil) consisting of a soil bacterium *Bacillus cereus* LB2 and an exclusive *Bacillus cereus* phage SWEP1 to illustrate how a single soil phage manipulates bacterial lysis and the associated necromass formation under different nutrient conditions and accociated changes on C and N dynamics. Results showed that the phage-induced bacterial lysis significantly increased the total amounts of necromass C and N, which was correlated to nutrient conditions. Bacteria populations were effectively eliminated by phages under eutrophic conditions, while the lysis rate of SWEP1 slowed down under oligotrophic conditions. The presence of phage clearly stimulated necromass accumulation, albeit with a reduced proportion of dissolved $NH_4^+$-N content from the supernatant. Therefore, phages may enhance microbial necromass formation, and actively contribute to soil organic matter (SOM) stabilization and C and N cycling in soil ecosystem.

**Keywords:** Soil phage, lysis, microbial necromass, nutrient cycling, SOM stabilization

**Highlights**

- Phages actively contribute to C and N retention in necromass
- Phage-mediated bacterial lysis increased C and N contents in necromass
- Eutrophic conditions facilitate phage-mediated bacteria elimination
- The lysis rate of phage slows down under oligotrophic conditions
- The contribution of bacterial necromass to SOM may be underestimated

Soil, as a highly heterogeneous and dynamic environment, hosts the highest biodiversity of Earth, including bacteria and viruses (Chevallereau et al., 2022; Kimura et al., 2008; Sun et al., 2021). It was estimated that the total number of virus-like particles (VLPs) in soils may reach $4.8 \times 10^{30}$ on Earth, accounting for about 10% of the total virus abundance ($> 10^{31}$) on Earth (Chevallereau et al., 2022). Bacteriophages (hereafter phages) are among the dominant groups of viruses, and contribute to manipulating bacterial community evolution and ecology via active infections (Braga et al., 2020; Rodriguez-Valera et al., 2009). In general, lytic phages infect and lyse bacteria cells (Cobián Güemes et al., 2016; Kuzyakov & Mason-Jones, 2018), resulting in the formation of phage-induced necromass. Thus, soil bacterium-phage interactions may play important functions in shaping the biogeochemical cycles of soil carbon (C) and nitrogen (N) and other elements across a broad range of environmental conditions (Kuzyakov & Mason-Jones, 2018; Pratama & van Elsas, 2018). While studies have shown that bacteria are crucial for soil C and N cycling, the functions of phages are often neglected (Kuzyakov & Mason-Jones, 2018) despite their abundant presence in most soils (Williamson et al., 2005).

According to a recently proposed concept of microbial necromass-associated soil C sequestration and N retention, the soil C and N pools are mainly derived from soil microbial necromass, accounting for over 50% of the total amount (Liang et al., 2019; Liang et al., 2020; Liang et al., 2017). Nevertheless, the neglection of the functions of phages onto the quantitative assessment of bacterial and phageal necromass dynamics, and impacts on soil organic matters (SOM) formation and subsequent soil C and N

retention capacity would likely result in a much-underestimated contribution of bacterial necromass onto the SOM patterns (Kuzyakov & Mason-Jones, 2018; Liang, 2020). We hypothesize that (1) phage-induced soil bacterial lysis increases soil necromass and thereby enhances soil C and N retention capacity, and (2) phage-induced bacterial necromass formation and subsequent SOM accumulation depend on nutrient conditions that likely manipulate soil phage-bacterium interactions. To quantify the effects of phage on bacterial lysis and subsequent necromass formation under different nutrient conditions and impacts on C and N retention in necromass, we conducted a set of laboratory microcosm incubation experiments in identical tubes consisting of soluble nutrients and a pair of model soil bacterium *Bacillus cereus* LB2 and phage SWEP1. Notably, our experiment was conducted in microcosms with cell/phage suspension and did not contain actual soil. The inoculum microorganisms all were isolated from agricultural soils (Mollisols) collected from the Lishu field experiment station of China Agricultural University (43°16′40.4″N，124°26′16.8″E), Lishu County, Jilin Province, China (Ruan et al., 2021). Thereinto, *B. cereus* LB2 bacteriophage, SWEP1, was isolated using *B. cereus* LB2 as the host bacterium, which was also isolated from the same soil sample (Ruan et al., 2021). Four sets of experiments were performed under oligotrophic (saline water: 0.9% NaCl solution) and eutrophic (LB fluid medium: consisting of 10.00 g/L Tryton, 5.00 g/L yeast extraction, 10.00 g/L NaCl) conditions marked as $LB2_{Olig}$, $LB2+P_{Olig}$, $LB2_{Eutr}$, and $LB2+P_{Eutr}$ that represent scenarios of ‘oligotrophic and phage absence’, ‘oligotrophic and phage presence’, ‘eutrophic and phage absence’, ‘eutrophic and

phage presence', respectively. The initial abundance of the *B. cereus* LB2 was $3.53\times10^7$ cfu $mL^{-1}$, and the initial abundance of the phage SWEP1 was $5.47\times10^7$ pfu $mL^{-1}$. All the experiments were conducted at 28℃, and samples were collected at 12 h after incubation for analysis. Thereinto, the live bacteria in all treatments ($LB2_{Olig}$, $LB2_{Eutr}$, $LB2+P_{Olig}$, and $LB2+P_{Eutr}$, although no live bacteria were detected in $LB2+P_{Eutr}$ treatment) were disrupted and killed by an Ultrasonic Homogenizer (JY92-IIDN, SCIENTZ, Inc., Ningbo, China) so that to separate and collect the mixed samples including solid residues (mainly containing residual medium, bacterial and phageal necromass) and liquid supernatants (mainly consisting of residual medium and intracellular substances released by cell disruption). Upon collection, we cleaned the solid residues three times with sterile water for eliminating the residual media on the solid surfaces, and then samples were centrifugated (Centrifuge 5415D, Eppendorf, Germany) at 4000×*g* for 10 mins to separate and collect the purified liquid supernatants, bacterial and phageal necromass. The $NH_4^+$-N concentrations in the initial medium, residual medium after culture, liquid supernatants were simultaneously measured employing a Seal AA3 Auto Analyzer (Seal Analytical, Inc., Mequon, WI, USA). The factual phage-induced $NH_4^+$-N concentration in the liquid supernatant was calibrated and calculated by subtraction. The total C ($C_{total}$, %) and total N ($N_{total}$, %) contents in the bacterial and phageal necromass were determined via combustion method by using an Elemental Analyzer (Vario EL III, Elementar, Langenselbold, Germany) (Wu et al., 2019). The total residues mass (including purified bacterial and phageal necromass) was weighed with an electronic balance

(±0.0001 g, QUINTIX224-1CN, Sartorius, Germany) after oven-drying upon collection. Bacterial and phage abundances were quantified using the double-layer agar plating counting method (Ruan et al., 2021; Yu et al., 2021). One-way ANOVA was applied to evaluate the effects of phage and nutrients condition on bacterial lysis and subsequent necromass formation using SPSS v. 21.0 (IBM Corp., Armonk, NY, USA). All figures were plotted using the Origin Pro ver. 9.3 (Origin Lab Corp., Northampton. MA, USA).

Figure 1A shows that living bacterial cell numbers increased over incubation time in the absence of the phage, which otherwise decreased at the presence of the phage, under both oligotrophic and eutrophic nutrient conditions ($p < 0.05$). The phage effect can be attributed to the killing and lysis of infected bacteria by the phage (Chevallereau et al., 2022). The magnitude of the treatment effects, however, were very different for the two nutrient conditions (Figure 1A, $p < 0.05$). In the absence of phage, bacterial cell numbers increased only slightly under the oligotrophic conditions, while they multiplied by three orders of magnitude under the eutrophic conditions. Conversely, the initial bacterial cell numbers were decreased by only about one-half by the inoculated phages, whereas the bacteria were eliminated below detection level in the eutrophic treatment. In contrast, no phages were detected out at the end of the experiment under the oligotrophic conditions, while their abundance increased by around two orders of magnitude under the eutrophic conditions (Figure 1B). These results indicate that phage lysis is much stronger and faster in environments rich in nutrients and energy, whereas oligotrophic conditions do not only limit bacterial

growth but also phage activity and the associating bacterial lysis (Chevallereau et al., 2022). The absence of phage at the end of the oligotrophic experiment scenarios suggests that either the initial inoculum phages infected but did not lyse bacteria, or they indeed caused bacterial lysis at the beginning of incubation. Nevertheless, the oligotrophic condition likely suppressed sufficient multiplication of the starved bacteria that eventually caused population elimination and thereby reduced phage infectivity (Brum et al., 2015; Brum & Sullivan, 2015; Williamson et al., 2017). Nevertheless, further investigation is needed to determine if the phage simply switched to a lysogenic-biased state coexisting with the host bacterium or the phage lysis rate was severely inhibited under oligotrophic conditions. Our findings show that the phage lysis rates can be significantly increased and thus be much higher under eutrophic than under oligotrophic conditions, suggesting that nutrient conditions may manipulate phage activities and phage-bacterium interactions in field soil situations (Safari et al., 2020). The negative relationship between bacterial and phage abundances (Figure 1) confirms that the interactions between *Bacillus cereus* LB2 and lytic phage SWEP1 were dominated by phage-induced bacterial lysis (Safari et al., 2020; Williamson et al., 2017).

The presence of phage significantly increased the $C_{total}$ and $N_{total}$ contents in the bacterial (and phageal) necromass (Figure 2), albeit it was modest in magnitude (19.8% - 47.4 % increase in $C_{total}$, 43.6% - 57.7 % increase in $N_{total}$). However, in contrast to the distinct influence of nutrient condition on bacterial and phageal abundances, its effects on the $C_{total}$ and $N_{total}$ contents did not differ by orders of

magnitude. In addition, the dissolved $NH_4^+$-N contents in the presence of phage under both eutrophic and oligotrophic conditions were significantly lower as compared with those of no-phage scenarios (Figure S1A). In contrast, the residues mass (mainly including the bacterial and phageal necromass) in the presence of phage under oligotrophic conditions increased by 160.9% as compared with those of none-phage scenarios, which was otherwise of a significant reduction of 68.8% for eutrophic ones (Figure S1B). Bacteria growth requires nutrient and energy which were scarce in oligotrophic environment. The presence of phages would likely kill the cells and release the intracellular dissolved nutrients, fostering the subsequent bacterial growth and producing residues mass. The results imply that the presence of phage may increase microbial residual mass (including bacterial and phageal necromass) by sufficiently recycling of intracellular dissolved nutrients in oligotrophic environments, that otherwise reduce the residual mass accumulation under eutrophic conditions (Feiner et al., 2015).

Collectively, our findings verified the hypothesis that phage-induced soil bacterial lysis manipulates microbial necromass formation and subsequent transformation and thereby, enhance soil C and N retention capacity. Most notably, this is a first attempt to quantify how phage-bacterium interactions function to regulate soil C and N retention that will provide a benchmark linking soil phage activity with C and N cycling. The soil environment is highly heterogeneous and dynamic, hence the spatial structure and heterogeneity could fundamentally alter the way phage-host interactions play out. Additionally, soil microbial necromass would be

likely decomposed by unaffected bacteria or fungi in a large part (Chevallereau et al., 2022). Nevertheless, the underlying mechanisms and functioning pathways are unclear, and urgently require in-depth investigations. In addition, soil phageal infection and lysis processes actively contribute to the enhanced microbial necromass formation (Cobián Güemes et al., 2016; Kuzyakov & Mason-Jones, 2018), as phages often reproduce themselves inside the host cells via utilizing the intracellular nutrients of lysed bacterial cells that likely stimulates SOM accumulation and turnover (Liang et al., 2020; Zhu et al., 2020). Thus, it may change $C_{total}$ and $N_{total}$ contents in the resulting necromass by selectively enriching these two elements. The underlying processes involved in bacterial (and phageal) necromass formation and SOM transformation, and impacts on global biogeochemical cycling and SOM pools need further study.

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**Author Contributions**

GW and HQW conceived the research and designed the methodology and experiments. HQW and SCW performed the experiments and the statistical analyses.

All authors analyzed and interpreted the data, all authors contributed to the writing of the paper, and all authors reviewed and approved the final version of the paper. GW initiated the project and GWC coordinated it.

## REFERENCES


Braga, L. P. P., Spor, A., Kot, W., Breuil, M., Hansen, L. H., Setubal, J. C., & Philippot, L. (2020). Impact of phages on soil bacterial communities and nitrogen availability under different assembly scenarios. *Microbiome*, *8*, 52.

Brum, J. R., Ignacio-Espinoza, J. C., Roux, S., Doulcier, G., Acinas, S. G., Alberti, A., ... Sullivan, M. B. (2015). Patterns and ecological drivers of ocean viral communities. *Science*, *348*(6237), 1261498.

Brum, J. R., & Sullivan, M. B. (2015). Rising to the challenge: accelerated pace of discovery transforms marine virology. *Nature Reviews Microbiology*, *13*(3), 147-159.

Chevallereau, A., Pons, B. J., van Houte, S., & Westra, E. R. (2022). Interactions between bacterial and phage communities in natural environments. *Nature Reviews Microbiology*, *20*(1), 49-62.

Cobián Güemes, A. G., Youle, M., Cantú, V. A., Felts, B., Nulton, J., & Rohwer, F. (2016). Viruses as winners in the game of life. *Annual Review of Virology*, *3*(1), 197-214.

Feiner, R., Argov, T., Rabinovich, L., Sigal, N., Borovok, I., & Herskovits, A. A. (2015). A new perspective on lysogeny: prophages as active regulatory switches of bacteria. *Nature Reviews Microbiology*, *13*(10), 641-650.

Kimura, M., Jia, Z., Nakayama, N., & Asakawa, S. (2008). Ecology of viruses in soils: Past, present and future perspectives. *Soil Science and Plant Nutrition*, *54*(1), 1-32.

Kuzyakov, Y., & Mason-Jones, K. (2018). Viruses in soil: Nano-scale undead drivers of microbial life, biogeochemical turnover and ecosystem functions. *Soil Biology and Biochemistry*, *127*, 305-317.

Liang, C. (2020). Soil microbial carbon pump: Mechanism and appraisal. *Soil Ecology Letters*, *2*(4), 241-254.

Liang, C., Amelung, W., Lehmann, J., & Kästner, M. (2019). Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology*, *25*(11), 3578-3590.

Liang, C., Kästner, M., & Joergensen, R. G. (2020). Microbial necromass on the rise: The growing focus on its role in soil organic matter development. *Soil Biology and Biochemistry*, *150*, 108000.

Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, *2*(8), 17105.

Pratama, A. A., & van Elsas, J. D. (2018). The 'neglected' soil virome – potential role and impact. *Trends in Microbiology*, *26*(8), 649-662.

Rodriguez-Valera, F., Martin-Cuadrado, A. B., Rodriguez-Brito, B., Pasic, L., Thingstad, T. F., Rohwer, F., & Mira, A. (2009). Explaining microbial population genomics through phage predation. *Nature Reviews Microbiology*, *7*(11), 828-836.

Ruan, C., Niu, X., Xiong, G., Chen, G., Wu, H., Ma, Z., ... Wang, G. (2021). Phenotypic and genotypic characterization of the new Bacillus cereus phage SWEP1. *Archives of Virology*, *166*(11), 3183-3188.

Safari, F., Sharifi, M., Farajnia, S., Akbari, B., Karimi Baba Ahmadi, M., Negahdaripour, M., & Ghasemi, Y. (2020). The interaction of phages and bacteria: the co-evolutionary arms race. *Critical Reviews in Biotechnology*, *40*(2), 119-137.

Sun, Y., Sun, M., Chen, G., Chen, X., Li, B., & Wang, G. (2021). Aggregate sizes regulate the microbial community patterns in sandy soil profile. *Soil Ecology Letters*, 3, 313-327.

Williamson, K. E., Fuhrmann, J. J., Wommack, K. E., & Radosevich, M. (2017). Viruses in soil ecosystems: An unknown quantity within an unexplored territory. *Annual Review of Virology*, *4*(1),

201-219.

Williamson, K. E., Radosevich, M., & Wommack, K. E. (2005). Abundance and diversity of viruses in six Delaware soils. *Applied and Environmental Microbiology*, *71*(6), 3119-3125.

Wu, H., Du, S., Zhang, Y., An, J., Zou, H., Zhang, Y., & Yu, N. (2019). Effects of irrigation and nitrogen fertilization on greenhouse soil organic nitrogen fractions and soil-soluble nitrogen pools. *Agricultural Water Management*, *216*, 415-424.

Yu, Z., Schwarz, C., Zhu, L., Chen, L., Shen, Y., & Yu, P. (2021). Hitchhiking behavior in bacteriophages facilitates phage infection and enhances carrier bacteria colonization. *Environmental Science & Technology*, *55*(4), 2462-2472.

Zhu, X., Jackson, R. D., DeLucia, E. H., Tiedje, J. M., & Liang, C. (2020). The soil microbial carbon pump: From conceptual insights to empirical assessments. *Global Change Biology*, *26*(11), 6032-6039.

**Figure Captions**

**Figure 1** Changes in bacteria (A) and phage (B) abundances after 12 h incubation at 28 ℃ under oligotrophic (Olig) and eutrophic (Eutr) conditions. LB2 represents the tested bacteria (*Bacillus cereus* LB2). P indicates the tested phage SWEP1 corresponding to LB2. LB2+P represents the treatment that SWEP1 was inoculated into LB2. *, $p < 0.05$; **, $p < 0.01$.

**Figure 2** Changes in total carbon (A) and nitrogen (B) contents of the bacterial + phageal necromass after 12 h incubation at 28 ℃ under oligotrophic (Olig) and eutrophic (Eutr) conditions. LB2+P represents the treatment in which *Bacillus cereus* LB2 was inoculated with the phage SWEP1. LB2 represents the control treatment without inoculation. P indicates the tested phage SWEP1 corresponding to LB2. Asterisks (*) indicate significant effects ($p < 0.05$).

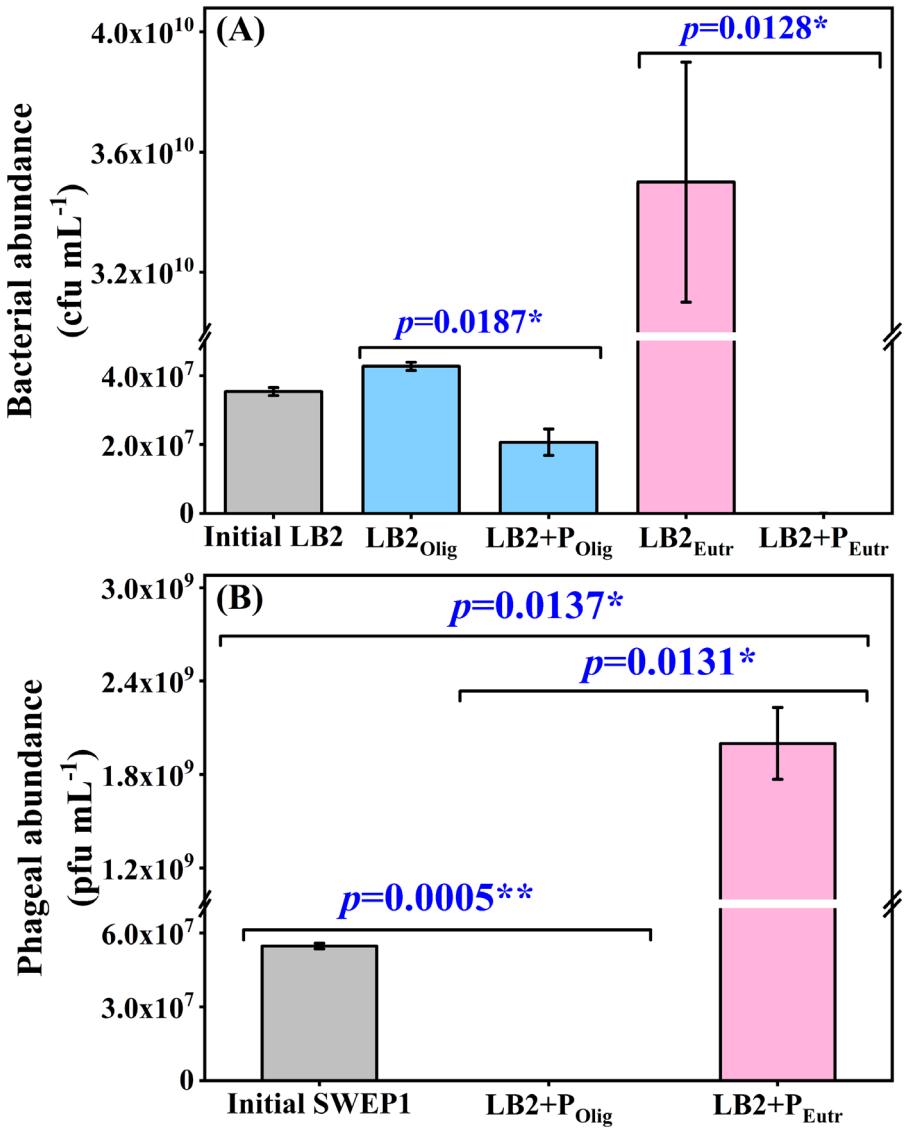


**Figure 1**

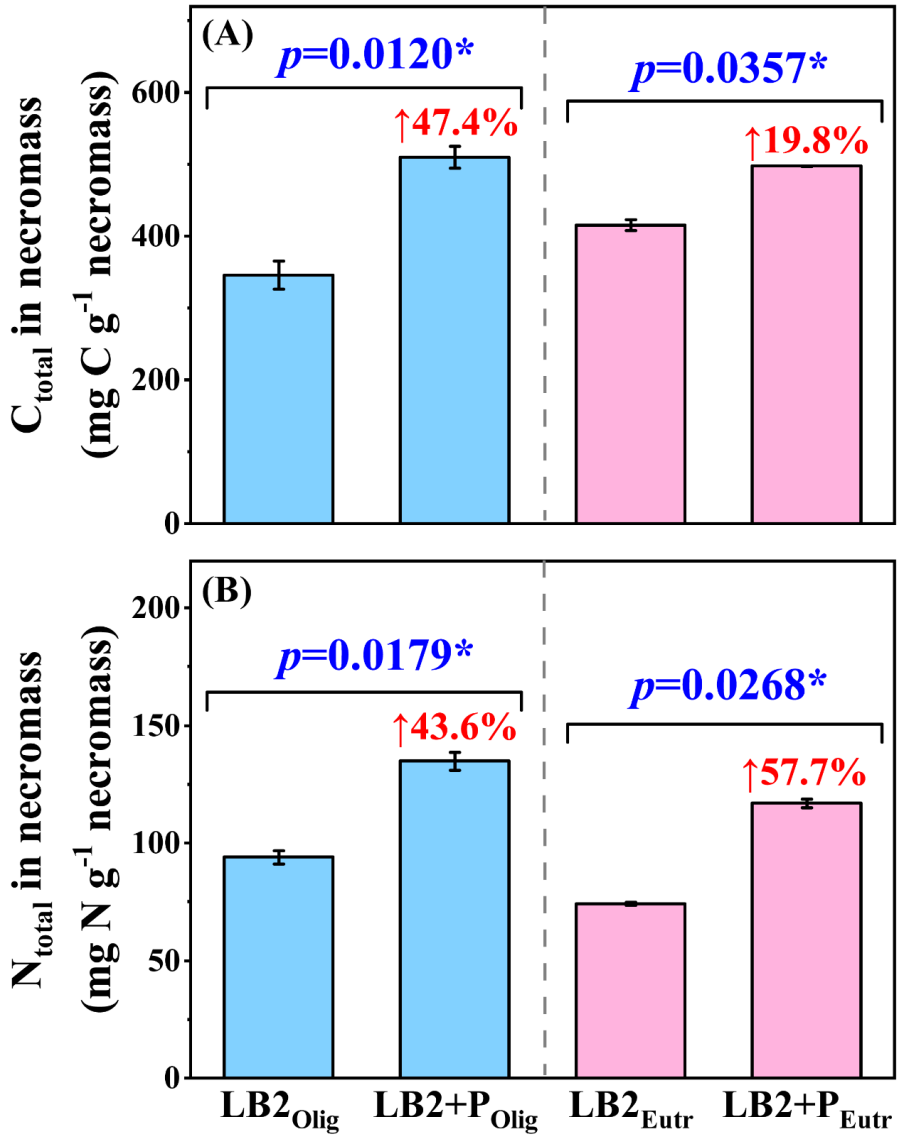


**Figure 2**