

Effects of Exogenous Phosphorus Input on Soil Carbon Pool Availability and Turnover Characteristics in Agricultural Wetlands: Postprint

Authors: Sun Xingzhao, Shen Jianguo, Zhong Wang, Liu Meng, Lou Liping, Yue Chunlei, Zhijian Zhang

Date: 2017-11-10T00:00:00+00:00

Abstract

By employing the technical approach of adding calcium superphosphate at rates equivalent to 0-60% of the native soil total phosphorus (TP) combined with laboratory incubation, this study investigated the dynamic effects of exogenous phosphorus input on the availability and turnover characteristics of the carbon pool in agricultural wetland soils. The results demonstrated that with increasing exogenous phosphorus input levels, soil dissolved organic carbon (DOC) and microbial biomass carbon (MBC) contents increased, whereas soil total organic carbon (TOC) content decreased, with a maximum reduction of 23%. Among the labile organic carbon fractions, exogenous phosphorus input exerted the most pronounced effect on highly labile organic carbon (HLOC), with HLOC content under the 60% phosphorus input treatment being 54% higher than that of the control without exogenous phosphorus addition. Readily oxidizable organic carbon (ROC) content decreased with increasing exogenous phosphorus input levels, with a maximum reduction of 22%; moderately labile organic carbon (MLOC) and labile organic carbon (LOC) contents remained unchanged. Regarding carbon-cycle-related enzymes, the activities of α -glucosidase (G) and cellobiohydrolase (CBH) both increased significantly with increasing exogenous phosphorus input levels, whereas dehydrogenase (DH) activity showed no significant variation. Furthermore, soil carbon mineralization intensity and potential mineralization potential were positively correlated with soil TP following exogenous phosphorus input. Collectively, exogenous phosphorus input significantly enhanced the availability and turnover rate of the wetland soil carbon pool.

Full Text

Effect of Exogenous Phosphorus Input on the Availability and Turnover Characteristics of Soil Carbon Pool in Agro-riparian Wetlands

SUN Xingzhao¹, SHEN Jianguo², WANG Zhong², LIU Meng¹, LOU Liping¹, YUE Chunlei³, ZHANG Zhijian¹, *

¹College of Environment and Resources, Zhejiang University, Hangzhou 310058, China

²Yuhang District Agricultural Ecology and Plant Protection Management Station, Hangzhou 311100, China

³Institute of Ecology, Zhejiang Academy of Forestry, Hangzhou 310023, China
Gusheng Agricultural Science and Technology Limited Company, Hangzhou 311108, China

*Supported by the National Natural Science Foundation of China (41373073, 41371447) and Zhejiang Province Science and Technology Project (2015F5002)

**Corresponding author, E-mail: zhangzhijian@zju.edu.cn

Abstract

Exogenous nutrients significantly influence wetland ecosystems, particularly the invasion of exogenous nitrogen (N) and phosphorus (P), which affects the carbon cycle in wetland soils and subsequently alters the global carbon cycle across the soil-atmosphere continuum. While several studies have examined exogenous N, research on exogenous P remains limited. To explore how exogenous P input affects the availability and turnover characteristics of soil carbon pools in wetlands, we designed an experiment simulating dynamic exogenous P accumulation by adding superphosphate at gradient rates of 0 (P-0), 5% (P-5), 10% (P-10), 20% (P-20), 30% (P-30), and 60% (P-60) relative to the initial total phosphorus (TP) content of sampled soils. Changes in soil carbon composition characteristics and biochemical P indicators were analyzed using conventional biochemical testing methods, with Pearson correlation analysis integrating multiple factors.

The results showed that soil TP and Olsen-P contents strictly followed the superphosphate loading rates, with notable increases in microbial biomass phosphorus (MBP). Acid phosphatase (AcP) activity was significantly suppressed by P addition. Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) increased with rising exogenous P inputs, while total organic carbon (TOC) decreased by up to 23%. Among active organic carbon fractions, highly labile organic carbon (HLOC) showed the most significant response, being 54% higher under P-60 treatment than the control. Recalcitrant organic carbon (ROC) content decreased with increasing exogenous P input, with a maximum reduction of 22%, while mid-labile organic carbon (MLOC) and labile organic

carbon (LOC) remained largely unchanged. Activities of α -1,4-glucosidase (G) and cellobiohydrolase (CBH) increased significantly with exogenous P levels, whereas dehydrogenase (DH) activity showed no significant change. Soil carbon mineralization intensity and potential mineralization were positively correlated with soil TP after exogenous P addition. Overall, exogenous P input significantly increased the availability and turnover rate of wetland soil carbon pools.

Keywords: Exogenous phosphorus; Agro-riparian wetland; Soil carbon pool; Carbon availability; Carbon turnover

Introduction

The agro-riparian wetlands of the Taihu Lake Basin represent an important agricultural economic region in China, characterized by dense population, prosperous economy, and high agricultural intensification. These wetlands serve as critical linkages between farmland and downstream water bodies, intercepting nutrient losses from cultivated soils and alleviating pollution loads in downstream waters while playing a vital role in global carbon storage. Due to slow organic matter degradation and high plant productivity, wetlands form significant carbon sinks in the earth's surface system, absorbing atmospheric CO₂ and CH₄ and mitigating global climate change. Consequently, wetlands have become a research hotspot because of their substantial carbon storage capacity.

Current research on wetland ecosystem carbon cycling has examined carbon pool composition and relationships with influencing factors, including how nutrient inputs affect wetland soil carbon sink functions. Studies by Peijl et al. demonstrated that increased nutrient input in floodplains enhances biomass production and soil organic matter accumulation. Liu et al. reported that exogenous N and P inputs increase soil organic carbon mineralization rates and accelerate the loss of soluble carbon components under flooded conditions. Dou et al. and Wang et al. found that nitrogen addition or deposition promotes carbon input and output in wetland soils, increasing the activity of soil carbon fractions. However, most research has focused on nitrogen and organic carbon changes, lacking comprehensive studies on how nutrient inputs affect overall soil carbon pool characteristics. In-depth reports on how exogenous phosphorus specifically affects the availability and turnover characteristics of wetland soil carbon pools remain scarce.

This study used wetland soils from rice cultivation areas in the southern Taihu Lake Basin as research objects. By adding phosphorus and conducting laboratory incubations, we simulated the process of agricultural non-point source phosphorus entering wetland ecosystems to dynamically investigate how exogenous P affects soil carbon pool availability and biochemical turnover characteristics, providing scientific information to enrich understanding of wetland ecosystem responses to exogenous nutrient inputs.

Materials and Methods

1.1 Study Area Overview

The study area is located in the southern delta farming region of the Taihu Lake Basin (119°54 E, 30°19 N), which contains extensive rice cultivation areas converted from wetlands. The region has a subtropical monsoon climate with an average annual temperature of 16°C and annual precipitation of 1,380 mm, concentrated during the June-July rainy season. Recent farmland total phosphorus losses in this area approximate 1.6–6.9 kg · hm⁻², with wetlands intercepting most of this lost phosphorus as crucial linkages between farmland and downstream waters, leading to further phosphorus deposition in wetland soils. The experiment selected representative alluvial paddy soils (0–20 cm) as test soils, with basic physicochemical properties shown in .

1.2 Experimental Design

Using a soil sampler, we randomly collected 20 surface soil samples (0–20 cm) from wetlands, removed plant roots and rhizomes, thoroughly mixed them, and transported them to the laboratory. Fresh soil samples (8 kg each, 34.6% water content) were placed in simulated wetland columns with a soil layer depth of approximately 20 cm. The experiment established six phosphorus gradient treatments as the sole variable: 0 (P-0), 5% (P-5), 10% (P-10), 20% (P-20), 30% (P-30), and 60% (P-60) of the original TP content of the test wetland soil to simulate accumulation effects of exogenous phosphorus, with three replicates per treatment. Water-soluble superphosphate served as the exogenous P source, completely dissolved in deionized water and slowly and evenly applied to the soil columns without disturbing the soil layer. After 2 hours of static equilibrium, deionized water was added to maintain a 10 cm water layer above the soil. All columns were incubated at 25°C in darkness to avoid interference from other factors.

Starting from the initial incubation date, soil samples were collected every 6 months along an “S” shaped route at four points in each column from the 0–10 cm surface layer. The four subsamples were thoroughly mixed to represent each treatment, with three sampling events total. After each collection, water levels were replenished to maintain the 10 cm water layer, with no water added between samplings to simulate periodic wetland flooding. Each collected sample was divided into three portions: (1) fresh samples passed through a 10-mesh sieve, stored in plastic bags at 4°C, and analyzed for soil biological properties within one week; (2) fresh samples passed through a 10-mesh sieve immediately used for soil mineralization incubation experiments; and (3) air-dried samples passed through 20-mesh and 100-mesh sieves for determination of wetland soil phosphorus and carbon physicochemical indicators.

Soil carbon mineralization intensity was determined using indoor constant-temperature incubation-instrument methods. Fresh samples (20 g) that had passed through a 10-mesh sieve were evenly spread on the bottom of 250 mL incubation bottles, sealed with rubber stoppers, and incubated in darkness at approximately 25°C for 56 days. On days 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56, 30 mL of gas was extracted from each bottle and CO₂ concentration was measured using an HP4890D gas chromatograph to calculate soil carbon mineralization. After gas collection, bottles were opened for 20 minutes to replenish oxygen and equilibrate with ambient air before resealing.

1.3 Measurement Methods

Total phosphorus (TP) was determined using HClO₄-H₂SO₄ acid dissolution-molybdenum antimony colorimetry. Olsen-P was measured using the pH 8.5 NaHCO₃ method. Total organic carbon (TOC) was analyzed by K₂Cr₂O₇ volumetric oxidation with external heating. Dissolved organic carbon (DOC) was measured using a TOC analyzer (Shimadzu TOC-VCPH). Active organic carbon fractions [readily oxidizable organic carbon (ROC), highly labile organic carbon (HLOC), mid-labile organic carbon (MLOC), and labile organic carbon (LOC)] were determined using potassium permanganate oxidation. Microbial biomass phosphorus (MBP) and microbial biomass carbon (MBC) were measured using chloroform fumigation-extraction, with conversion coefficients of 0.40 and 0.45, respectively.

Soil phosphorus-related enzyme (acid phosphatase, AcP) and carbon-related enzymes (α-1,4-glucosidase, G; cellobiohydrolase, CBH) activities were measured using microplate fluorometric assays, while dehydrogenase (DH) activity was determined using triphenyltetrazolium chloride colorimetry. Enzyme activity calculations followed DeForest's methods.

1.4 Data Analysis

Experimental data were analyzed using Microsoft Office 2013 and SPSS 19.0 for statistical analysis and significance testing, with OriginPro 9.0 used for plotting. The cumulative CO₂-C release process followed a first-order kinetic equation, expressed as:

$$MATH_0$$

where C_t is the cumulative CO₂ released from soil after time t (days) ($\text{mg} \cdot \text{kg}^{-1}$), C_0 is the potentially mineralizable active organic carbon in soil ($\text{mg} \cdot \text{kg}^{-1}$), k is the turnover rate constant of the active organic carbon pool (d^{-1}), and t is incubation time (days).

Results

2.1 Effects of Exogenous Phosphorus Input on Soil Phosphorus Properties

As shown in [Figure 1: see original paper], within the same treatment group, wetland soil TP content showed no significant change over incubation time, while MBP and Olsen-P contents in high-P treatments exhibited decreasing trends. However, at each sampling event, overall contents of TP, MBP, and Olsen-P increased with exogenous P input levels, with the most significant increases in the P-60 treatment ($P < 0.05$). After the entire incubation period, TP content in the control (P-0) remained essentially unchanged compared to the original wetland soil TP content, while the other five treatments increased by approximately 5%, 10%, 20%, 30%, and 60%, respectively, with significant differences among groups ($P < 0.05$). This indicates that phosphorus, as a relatively stable element in soil, produces obvious cumulative effects in input systems. MBP and Olsen-P followed similar trends to TP, but over incubation time, the P-60 treatment showed significant decreases in both MBP and Olsen-P. For Olsen-P, this may be because phosphorus-rich soils have higher active phosphorus release rates under flooded conditions, indicating greater phosphorus output risk. For MBP, in the absence of other influencing factors, the enhanced microbial activity stimulated by exogenous P input would gradually weaken as substrates are consumed, and the immobilized microbial biomass would turnover back into the soil medium through microbial physiological metabolism.

When soil phosphorus is limited, microorganisms secrete phosphatase to stimulate the transformation of organic phosphorus to inorganic phosphorus, so microbial demand for phosphorus can be reflected by phosphatase activity changes. In this experiment, the added superphosphate was inorganic phosphorus that could be directly absorbed by microorganisms, requiring no phosphatase participation while directly increasing soil available phosphorus content and alleviating phosphorus limitation. Consequently, acid phosphatase (AcP) activity was significantly suppressed with increasing exogenous P input ($P < 0.05$). Meanwhile, differences among treatment groups decreased over time, suggesting that phosphatase activity differences caused by phosphorus input would gradually diminish.

2.2.1 Total Organic Carbon

As shown in [Figure 2: see original paper], wetland soil TOC content generally decreased with increasing exogenous P input levels. After 6 months of incubation, TOC contents in each treatment decreased by 3%, 4%, 6%, 11%, and 13% compared to the control, with no significant differences between the control and low-P treatments (P-5, P-10), but significant differences from high-P treatments (P-20, P-30, P-60) ($P < 0.05$). Similar trends were observed after 12 and 18 months. Additionally, TOC content in the same P treatment decreased significantly over time, with control TOC contents at 12 and 18 months decreasing

by 4% ($P < 0.05$) and 10% ($P < 0.05$), respectively, compared to 6 months.

2.2.2 Dissolved Organic Carbon

As shown in [Figure 3: see original paper], soil DOC content increased significantly with exogenous P input levels within the same sampling period ($P < 0.05$), while overall DOC content decreased over incubation time. During the entire incubation period, differences among treatment levels were most significant at 12 months ($P < 0.05$), with DOC contents increasing by 3%, 6%, 9%, 11%, and 19% compared to the control. By 18 months, inter-treatment differences had diminished, but DOC contents in P-30 and P-60 treatments remained 1.17 and 1.22 times the control ($P < 0.05$). Compared to the 6-month P-0 treatment, control DOC contents decreased by 8% and 30% at 12 and 18 months, respectively ($P < 0.05$).

2.2.3 Microbial Biomass Carbon

As shown in [Figure 4: see original paper], soil MBC content increased significantly with exogenous P input levels at each sampling ($P < 0.05$), but decreased over incubation time within the same treatment. At 6 months, MBC contents in each treatment were 1.31, 1.64, 1.47, 1.55, and 1.69 times the control, decreasing to 1.17–1.40 times the control by the end of the incubation period, with the increasing trend slowing. Compared to the 6-month P-60 treatment, control MBC contents decreased by 11% and 22% at 12 and 18 months, respectively ($P < 0.05$).

2.2.4 Active Organic Carbon Fractions

After exogenous P addition, the four organic carbon fractions ranked as ROC > LOC > HLOC > MLOC ([Figure 5: see original paper]). With increasing exogenous P input, MLOC and LOC contents remained essentially unchanged, HLOC content increased significantly during early incubation ($P < 0.05$), while ROC content continuously decreased ($P < 0.05$). At 6 months, HLOC contents were 1.14, 1.17, 1.43, 1.38, and 1.54 times the control, while ROC decreased to 98.5%, 94.6%, 91.9%, 82%, and 78% of the control. After the entire incubation period, compared to 6 months, control HLOC content decreased by approximately 8% ($P < 0.05$), while P-60 HLOC content decreased by about 31% ($P < 0.05$). Changes in HLOC at 12 and 18 months were not significant with increasing P input. Meanwhile, ROC content in the control at 18 months decreased by only about 1.1% compared to 6 months, showing little change, while ROC in P-60 decreased by approximately 16% ($P < 0.05$).

2.2.5 Organic Carbon Mineralization Characteristics

As shown in [Figure 6: see original paper], during three 56-day incubation periods, organic carbon mineralization rates in all treatments peaked on day 5, then

gradually decreased over time, stabilizing during the final two weeks. Mineralization rate trends at 6 and 12 months were consistent, while rates at 18 months were significantly lower, with maximum values reduced to half of previous peaks. Except for P-5 and P-10 treatments, which had higher mineralization rates than P-0 only during the first week before converging as active carbon fractions were consumed, all other treatments showed significantly higher mineralization rates than the control ($P < 0.05$).

In all three soil carbon mineralization experiments, cumulative mineralization increased significantly with exogenous P input levels ($P < 0.05$). At 6 months, cumulative mineralization increased by 2.7%, 9.5%, 24%, 35%, and 49% compared to the control. Inter-treatment differences decreased with incubation time, and by 18 months, cumulative mineralization increased by only 2.2%, 6.2%, 15%, 26%, and 36% compared to the control.

First-order kinetic equation fitting of cumulative mineralization (see) showed that at 6 months, potential mineralization (C) ranged from 40.9–66.8 $\text{mg} \cdot \text{kg}^{-1}$, increasing significantly with exogenous P input ($P < 0.05$), with C values being 0.99, 1.21, 1.28, 1.45, and 1.62 times the control. However, the mineralization constant k showed no regular pattern. At 12 months, C decreased to 45.2–58.6 $\text{mg} \cdot \text{kg}^{-1}$ but remained significantly higher than the control ($P < 0.05$). At 18 months, treatment differences diminished, with P-5 and P-10 showing slightly lower C than the control, while P-20, P-30, and P-60 remained significantly higher ($P < 0.05$).

2.2.6 Carbon Cycle Enzyme Activities

Carbon cycle enzymes G and CBH, associated with macromolecular cellulose degradation, dynamically reflect soil carbon pool changes. G, as an extracellular enzyme limiting macromolecular organic matter degradation, can increase small molecular soluble substrate content. As shown in , exogenous P input significantly enhanced G activity ($\text{LRR}^* > 0$). At 6 months, G activity reached its maximum across all samplings, with significant treatment differences ($P < 0.05$) and RR values of 1.09, 1.31, 1.57, 1.66, and 1.77 times the control. Inter-treatment differences weakened over time, though P-60 RR values remained 16% higher than the control at 18 months. CBH activity also increased with exogenous P input ($\text{LRR}^* > 0$), but treatment differences were less significant than for G and diminished over time. Unlike G and CBH, DH is a redox enzyme reflecting overall microbial metabolic activity. DH activity remained essentially unchanged compared to the control ($\text{RR} = 1$), indicating that exogenous P input did not significantly affect soil DH activity.

Discussion and Conclusions

As extensive wetlands have been reclaimed for agriculture, nutrients applied in agricultural activities (N, P, etc.) enter wetland ecosystems through surface

runoff and atmospheric precipitation, disrupting the original balance of natural wetlands. Our results demonstrate that increasing exogenous P input enhances wetland soil carbon pool availability and accelerates turnover rates, consistent with reports by DeBusk et al. and Liu et al. that exogenous phosphorus promotes organic carbon mineralization and turnover in wetland soils.

Throughout the dark, constant-temperature incubation, soil TOC content decreased significantly with both increasing exogenous P input and incubation time, indicating that exogenous P input can increase consumption of the soil carbon pool. Without exogenous carbon input, limited available carbon components in the initial soil were rapidly decomposed by activated microorganisms following exogenous P addition, with decomposition showing time-cumulative effects that strengthened over time. Soil DOC and MBC contents increased with exogenous P input but decreased substantially over incubation time, suggesting that exogenous P input promotes transformation of the soil carbon pool from a stable to an unstable state, enhancing conversion of soil organic matter to microbial biomass. This DOC likely originated from humus components in soil organic matter. Under conditions without exogenous carbon input, the accumulation intensity of MBC induced by exogenous P input decreased, and microbial dissimilation rapidly degraded unstable carbon components.

Analysis of active carbon fractions revealed that HLOC content increased while ROC content decreased with exogenous P input, with overall HLOC content decreasing significantly and ROC content decreasing slightly. This indicates that exogenous P input drives soil carbon pool transformation from stable to unstable states as an immediate feedback to rapidly changing environmental conditions. Without exogenous carbon input, soil active carbon fractions decomposed due to microbial dissimilation, with decomposition increasing with exogenous P input levels. Previous research has shown that HLOC is much more sensitive to soil carbon pool changes than ROC, and while ROC is a relatively stable component in soil carbon pools, exogenous P input still promoted ROC degradation. Soil LOC and MLOC were essentially unaffected by exogenous P input.

Studies have shown that organic matter is the main carbon source for soil respiration, with respiration rates increasing with organic matter content. Accelerated decomposition of soil organic matter due to improved phosphorus availability can increase CO₂ emissions. Ma et al. also demonstrated that long-term phosphorus fertilizer application significantly promotes soil organic carbon mineralization. In this study, the high exogenous P treatment (P-60) showed significantly higher organic carbon mineralization rates than the control, consistent with Aerts et al., who reported that small amounts of inorganic phosphorus have limited effects on soil carbon mineralization, but when baseline soil phosphorus is low, respiration intensity positively correlates with exogenous P input levels. Li et al. found that dynamic changes in soil organic carbon mineralization rates align with DOC content changes. In this study, differences in cumulative organic carbon mineralization among treatments induced by exogenous P input decreased over time, with significant reductions in mineralization across

all treatments, consistent with previous DOC content trends. Soil potential mineralization (C) increased with exogenous P input, with inter-treatment differences decreasing significantly over time, indicating that exogenous P input may increase the risk of carbon output from wetland soils, while cumulative effects of exogenous P input can increase total potential mineralization.

DOC analysis revealed that soil DOC content increased with exogenous P input, and as a microbial metabolic substrate, increased DOC content promotes microbial activity and synthesis of related enzymes. This study found that exogenous P input significantly enhanced G and CBH activities but did not significantly affect DH activity, differing from Dinesh et al., who reported that inorganic fertilizer addition significantly increased DH activity. However, other studies have indicated that inorganic fertilizer addition essentially does not change DH activity, and differences in soil type, topography, and local climate conditions may explain these varying results.

Pearson correlation analysis of all experimental data ([Figure 7: see original paper]) showed that soil TP, Olsen-P, and MBP were significantly negatively correlated with TOC and ROC ($P < 0.01$) but significantly positively correlated with unstable carbon fractions (MBC, DOC, HLOC) and cumulative CO₂ emission (C) ($P < 0.01$), indicating that exogenous P input reduces total wetland soil carbon, promotes formation of active carbon fractions, and accelerates soil carbon pool mineralization, causing substantial wetland carbon pool losses. Carbon cycle enzymes G and CBH were positively correlated with TP ($P < 0.05$) and significantly positively correlated with DOC and HLOC ($P < 0.01$), suggesting that exogenous P input increased G and CBH activities and synthesized new enzymes by increasing substrate—active carbon components of the soil carbon pool. Meanwhile, G and CBH were significantly negatively correlated with ROC ($P < 0.01$), indicating that activated carbon-related enzymes accelerated the conversion of macromolecular cellulose to highly labile carbon fractions.

In summary, exogenous P input increases soil carbon pool consumption, drives transformation from stable to unstable states, stimulates microbial activity, enhances enzyme activity, promotes synthesis of unstable carbon fractions, increases carbon pool availability, accelerates turnover rates, and ultimately increases the risk of carbon output from wetland soils.

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