

Sedimentary Organic Matter Load Influences the Ecological Effects and Potential Risks of Submerged Macrophyte Restoration Through Rhizosphere Metabolites

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Research Article

Keywords: sediment, submerged macrophyte, restoration, rhizosphere metabolites

Posted Date: July 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-676658/v1>

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Abstract

Aims Rehabilitation of submerged vegetation is one of the commonly used techniques for the ecological restoration of shallow lakes. The changes of pollution structure in sediments caused by plant recovery and the rhizosphere chemical process under different sediment organic matter (SOM) levels are theoretical basis for the rational application of plant rehabilitation technology in lake management.

Methods A circulating extraction system was designed for in situ collection of rhizospheric metabolites especially for the submerged plants. We explored how *Vallisneria natans* (*V. natans*) mediate the changes in sediment N and P through rhizospheric metabolites under low (4.94%) and high (17.35%) SOM levels.

Results By analysing 63 rhizospheric metabolites from *V. natans*, glucitol was found to be 146.82% lower in the low SOM than in the high SOM treatment. $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ increased by 57% and 68.39%, respectively, in the high SOM treatment, while approximately one-seventh Inorg-P was transferred from Fe/Al-P to Ca-P in the low SOM treatment. The metabolites lactic acid, 3-hydroxybutyric acid, and phosphoric acid mediated $\text{NH}_4\text{-N}$ accumulation. Additionally, 3-hydroxy-decanoic acid and adipic acid mediated the transformation of Fe/Al-P to Ca-P.

Conclusions The growth of *V. natans* significantly changed Inorg-N or Inorg-P fractions. The changes were SOM level-dependent and rhizosphere metabolites related. This study emphasised the benefit of *V. natans* rehabilitation at low SOM level. When restoring submerged macrophytes from high SOM sediment, care should be taken due to the release potential of labile N and P forms.

Introduction

Eutrophication caused by intensive anthropogenic disturbance has led to the loss of submerged macrophytes and toxic algal blooms, which negatively affect ecological function and service (Hilt et al., 2017). Nutrient loadings from surface runoff, urban wastewater discharge, and aquaculture sewage have become widespread problems causing organic matter enrichment in sediment (Lee et al., 2019, Tanentzap et al., 2019). The decline of submerged macrophytes, especially rooted plants, suppresses relevant ecosystem functions mediated by macrophytes, such as the control of sedimentary N and P metabolism (Soana et al., 2015, Han et al., 2018). This generates a positive feedback to eutrophication, as slower mineralization rates result in a net organic matter accumulation.

Rooted plants influence the physical and chemical characteristics of rhizosphere soil through a complex mixture of compounds released from roots, that is, root exudates, which have a significant impact on nutrient and trace element dynamics (Rugova et al., 2017). A total photosynthetically fixed C of up to 50% are transferred to the rhizosphere through root exudates, of which low molecular weight compounds include organic acids, sugars, amino acids, and other secondary metabolites (Vranova et al., 2013, Van Dam and Bouwmeester, 2016). Untargeted metabolomics is increasingly becoming an accepted method for identifying compounds that are secreted by plants (Van Dam and Bouwmeester, 2016), which is important for understanding belowground chemical communication. Related studies on the impacts

of ammonia retention, N cycle, P-mobilising, and soil zymography have been reported for their close association with rhizospheric metabolites by terrestrial plants (Coskun et al., 2017, Gusewell and Schroth, 2017, O'Sullivan et al., 2017, Zhang et al., 2019). Specific compounds deemed as biological nitrification inhibitors, such as sorgoleone, sakuranetin, and methyl 3-(4-hydroxyphenyl) propionate, have been identified and tested for their effectiveness in reducing N losses and pollution from agriculture (Coskun et al., 2017). Carboxylates from root exudates such as citrate, oxalate, and malate are usually reported as P-mobilising compounds (Dessureault-Rompere et al., 2007, Wang et al., 2015). Root exudates from three emergent plants significantly influence the abundance and community diversity of the denitrifier genes *nirK* and *nirS* in constructed wetlands (Wu et al., 2017). Inorg-P release induced by low molecular weight organic acids was soil type dependent, whereas Organ-P release was ascribed to their ability to mobilise labile P rather than their ability to chelate cations bound to P (Wang et al., 2015). Root exudate-mediated soil N and P changes have been reported for a long time, whereas much less is known about the effects of metabolic compounds from submerged macrophytes on the sediment N and P fractions, especially under different organic matter levels.

The poor recovery of aquatic plants is possibly caused by increasing organic matter accumulated on the top layer of the sediment (Pulido et al., 2011). An increase from 5% to 25% of total organic matter might be buried in sediment due to intensive anthropogenic activities (Tanentzap et al., 2019). NH_4^+ , NO_3^- , and NO_2^- flux were all influenced by sediment organic levels, in which NH_4^+ flux was the most significant (Soana et al., 2015). Formation of metal (Ca, Fe, or Al) phosphate precipitates is modulated by soil organic matter (Ge et al., 2019). Therefore, organic matter level is an important factor in sediment N and P fractions, and further investigation of the relevance of metabolic compounds during the macrophyte recovery process is still needed.

Rooted submerged macrophyte *Vallisneria natans* was selected as a case study due to its well-developed root system and widespread distribution in tropical and subtropical areas (Zhang et al., 2016). Although it is a commonly used species in lake and river rehabilitation, rhizospheric metabolism-mediated mechanisms of its impact on sediment is still unclear, and its applicability to different sediment organic matter (SOM) levels requires an in-depth theoretical basis. Therefore, the aim of this study was to explore the rhizospheric metabolites dynamic in submerged macrophytes, and the metabolism-mediated impact on N and P fractions in the sediment. The hypotheses are as follows: 1) different types of rhizospheric metabolites of *Vallisneria natans* (*V. natans*) can significantly distinguish plant growth stages and sediment organic matter levels, and 2) specific metabolites are responsible for the change in sedimentary N and P fractions.

Materials And Methods

Experimental setup and sampling

A collection system for rhizospheric metabolite compounds (**Fig. 1**) was developed, in particular for submerged macrophytes, including a main box structure, resin column, connection elements, and pump.

The main box was a 20 cm × 12 cm × 30 cm cubic structure made of polymethyl methacrylate. A division plate was designed to separate the plant chamber and the non-plant chamber. Two sand core funnels were fixed on the bottom of each chamber, and they were combined to one resin column through a Y-type triple-valve piston below the main box. Quartz sand (5 cm deep) was placed in each chamber, followed by lake sediment (8 cm deep). Bundles of *V. natans* specimens were transplanted above the sand core funnels. The teflon pipe and pump were connected to create a circulation loop from the rhizosphere to the resin column. For each metabolite sampling, the pump was turned on for 24 h and the flow rate was set to 20 mL min⁻¹. After cyclic extraction, the resin was eluted to obtain the desired compounds.

According to the Guidelines for the Protection and Management of Aquatic Sediment Quality by Persaud et al. (1993), an aquatic sediment level of 17.24% can be classified as severe, and such a sediment concentration of a compound would be detrimental to the majority of benthic species. Therefore, sediments with low and high organic matter levels (4.94% and 17.35%, respectively, as loss on ignition, LOI) were obtained from Maojiabu and Xilihu, two lake areas of West Lake, Hangzhou, China. Each sediment treatment was repeated for three collection devices (**Fig. 1**). The abbreviations L and H represent low and high SOM levels, respectively, and V and N represent the treatment with and without *V. natans*, respectively. Rhizospheric metabolic compounds were extracted every 10 days for a total of 7 times. Plant and sediment samples were taken before and after the 70-day incubation period.

Metabolites collection for GC–MS analysis

The XAD-4 resin from each column was first dried by vacuum filtration, followed by eluting with 300 mL of methyl alcohol. The eluent was dried using a rotary evaporator, and dissolved in 2 mL methyl alcohol. After centrifugation at 12,000 r/min for 5 min, the supernatant of the concentrated solution was transferred into a new tube, and 50 µg nonadecanoic acid methyl ester added as an internal standard. Samples were re-evaporated to dryness, and then dissolved in 100 µL pyridine for methoximation (70 °C, 2 h) with 20 mg/mL methoxyamine hydrochloride and derivatization (70 °C, 2 h) with 100 µL N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA). After centrifugation at 12,000 r/min for 5 min, the supernatant was ready for GC–MS analysis.

Gas chromatograph analysis was performed using an Agilent 6890N gas chromatograph (Agilent Technologies, USA), equipped with an Agilent 5793 inert MSD. A fused silica capillary column (HP-5MS, 0.25 mm × 30 mm × 0.25 µm) was employed. The GC oven temperature programme consisted of an initial temperature of 70 °C held for 1 min, with 2 °C/min increases to 120 °C, held for 1 min, then 4 °C/min increases to 220 °C, held for 1 min, and then 30 °C/min increases to 280 °C, held for 5 min. The injector temperature was 250 °C, set at an injected volume of 1 µL. Helium (purity > 99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The mass spectral library of the National Institute of Standards and Technology (NIST) in the U.S. was used as a reference. Peak areas were normalised by the peak area of the internal standard substance in each sample.

Plant morphological parameters analysis

Two bundles of *V. natans* specimens were transplanted in each plant chamber with the roots directly above the sand core funnel. A total of 36 single *V. natans* were physically labelled with nylon thread connected tags, and mixed with unlabelled plants. Each plant chamber was assigned 6 labelled plants, which were analysed for morphological parameters before and after the 70 days of incubation. They were scanned by Microtek ScanMaker i800 (Microtek, Shanghai, China), and the morphological parameters of the root and leaf, including total length and surface area, were calculated with LA-S Root Analysis software (WSeen, Hangzhou, China).

Measurements of the sediment N and P forms

The sediment samples were measured for N and P fractions before and after the 70-day incubation. The air-dried samples were ground into powder, after removing rocks, spiral shells, and plant matter, and the powder was sieved through a 0.15 µm mesh and collected for further analysis. TN was analysed using the persulfate digestion method (Qian et al., 1990). NH₄-N, NO₃-N, and NO₂-N contents were measured via extraction with potassium chloride solution and spectrophotometric methods (China Ministry of Environmental Protection, 2012). Sediment TP was determined using the ascorbic acid method after incubation at 450 °C and extraction with 3.5 M HCl. P fractions in the sediments were determined using the SMT harmonised protocol (Ruban et al., 2001). The operationally defined scheme was composed of five phosphorus forms: Fe/Al-P (NaOH-extractable P, P bound to Al, Fe, and Mn oxides and hydroxides), Ca-P (HCl-extractable P, P associated with Ca), Org-P (organic P), Inorg-P (inorganic P), and TP (total P).

Statistical analysis

Using the software package PASW Statistics 18.0, the paired sample t-test was used to analyse the significance of the morphological parameters of the roots and leaves. For each metabolite, the normalised peak area was expressed as peak area relative to the internal standard compound. Univariate analysis by means of three-way ANOVA was used to analyse any significant effects of time series, SOM level, plant, and their interactions on metabolic compounds. One-way ANOVA was used to analyse the significance of the sediment N and P forms. The Student–Newman–Keuls method was adopted for stepwise multiple comparisons.

Partial least squares discriminant analysis (PLS-DA) was performed with SIMCA 13.0 software, and the metabolite heat map was drawn with MeV 4.9.0 software. Key metabolites for differentiating the paired treatment groups were based on their variable importance in the projection (VIP) values (VIP > 1). Pearson's correlation analysis (between sedimentary variables and metabolic compounds) was conducted using PASW Statistics 18.0. Fig.s depicting line series, column plots, and a colour bubble map were created using OriginPro 9.0 software.

Results

Morphological acclimatization of V. natans

The *V. natans* specimens displayed remarkable differences in root and leaf development under low SOM and high SOM levels. They were stimulated at the low SOM level, but inhibited at the high SOM level. Below-ground tissues reproduced through stolon were observed only at the low SOM level. The total length of roots and leaves increased by 60.93% and 54.07%, respectively, after the incubation period at the low SOM level, whereas the two indicators (roots and leaves) decreased by 12.05% and 23.37% at the high SOM level (**Fig. 2a**). The total surface area of root and leaf increased by 54.35% and 79.05%, respectively, after the incubation period at the low SOM level, whereas the two indicators decreased by 38.35% and 45.69%, respectively, at the high SOM level (**Fig. 2b**).

An overview of rhizospheric metabolic compounds

In total, 79 putative metabolites were detected across all treatments. Among these, 63 compounds were identified and grouped into 18 subclasses and 7 descriptive classes, including 25 organic acids, 12 alcohols, 7 sugars, 9 amines, 5 phenolics, 3 phosphates, and 2 hydrocarbons (**Fig. 3**). Organic acids and alcohols were the main classes, accounting for 49.07% and 38.33% of the total abundance, respectively. Lactic acid, glycerol, 1,2-propanediol, and 2,3-butanediol were the dominant metabolites, accounting for 25.04%, 11.78%, 10.33%, and 7.78% of the total abundance, respectively. Univariate analysis indicated that categories of organic acids, alcohols, amines, and phosphates were significantly different by time series, SOM level, plant, or their interactive effects. Of the 63 compounds tested, 32—13 organic acids, 4 alcohols, 5 sugars, 5 amines, 3 phenolics, and 2 phosphates—proved significantly different either by main effect or interactive effect (**Tables S1–S3**). The total amount of detected metabolites was highest in the L-V group, followed by H-V, L-N, and H-N. Alcohols was the major contributor to the difference among these groups.

Effects of plant development stages on metabolic compounds

Compounds, their subclasses, and classes that significantly differed by time series, or interaction effects between time series and SOM level or plant, were displayed by the time series as the horizontal axis. Alcohols and organic acids were detected to be higher in the early stage, especially for the planted treatments (**Fig. 4a, b**). The normalised peak areas of alcohols and organic acids were 73.92% and 90.83% higher, respectively, in the period 10–30 d than in the period 40–70 d. Amines and phosphates were detected to be higher in the later stage (**Fig. 4c, d**). The normalised peak area of amines and phosphates were 39.24% and 526.42% higher, respectively, in the period 40–70 d than in the period 10–30 d.

As the most abundant organic acid subclass, hydroxy fatty acid showed a “V-type” fluctuation during the incubation period (**Fig. 5a**). Saturated fatty acids, dicarboxylic acids, and unsaturated fatty acids were detected to be higher in the early stage. The normalised peak areas of these 3 fatty acids were 92.30%, 24.92% and 191.69% higher, respectively, in the period 10–30 d than in the period 40–70 d (**Fig. 5b–d**). Saturated fatty acids and unsaturated fatty acids varied significantly between planted and non-planted treatments in the later and early stages, respectively. Saturated fatty acids were 6.14 times higher in the planted treatment than in the non-planted treatment on the 60th day. Unsaturated fatty acids were 2.19

times higher in the non-planted treatment than in the planted treatment on the 20th day. Enol and monosaccharide showed significant differences between treatments in the later stages (**Fig. 5e, f**). The normalised peak area of enol was 10.21 in the planted treatment on the 60th day, while no peak was detected for the non-planted treatment. Monosaccharide content was 8.03 times higher in the low SOM treatment on the 60th day. Amino acid was 1.05 times higher in the non-planted treatment in the period 10–50 d, whereas it was 0.66 times higher in the planted treatment in the period 60–70 d (**Fig. 5g**). Phosphoric acid represented a minor part of the total metabolites, and it was notably higher in the low SOM treatment on the 50th day (**Fig. 5h**).

Metabolic compounds were regrouped into early stage (10–30 d) and later stage (40–70 d) instead of 7 independent stages, due to the two-stage characteristics shown by the descriptive class and subclass. PLS-DA indicated that 22 compounds (VIP > 1) were significantly different between the early and later stages. Lauric acid, cinnamic acid, benzoic acid, myristic acid, and palmitoleic acid had the 5 highest VIP values, which contributed to the stage differences (**Fig. 6a**). Relative fold changes of each VIP compound were calculated as $(\text{Average}_{10-30d} - \text{Average}_{40-70d}) / \text{Average}_{10-70d}$. Eleven compounds were more abundant in the early stage than in the later stage, whereas the other 7 compounds showed the opposite trend. Specifically, the relative fold changes of 2,5-dimethyl-2,5-hexanediol, lauric acid, proline, and palmitoleic acid were higher than 150%, whereas the relative fold changes of ribose, pyroglutamic acid, glucitol, and glycine were lower than -150% (**Fig. 6a**).

Effects of SOM levels on metabolic compounds

Among all the compound subclasses, monosaccharide was 0.79 times higher in the high SOM treatment than in the low SOM treatment, and phosphoric acid was 2.44 times higher in the low SOM treatment than in the high SOM treatment (**Fig. S1**). Univariate analysis indicated that 4 compounds had significantly different organic matter (OM) levels (**Table S3**). A total of 20 compounds were extracted by PLS-DA, including the above 4 compounds. Relative fold changes of each VIP compound were calculated as $(\text{Average}_{\text{low SOM}} - \text{Average}_{\text{high SOM}}) / \text{Average}_{\text{total}}$. Twelve compounds were more abundant in the low SOM treatment than in the high SOM treatment, whereas the other 8 compounds showed the opposite trend. Glucitol was the most crucial compound that distinguished the metabolite profile between low and high SOM levels, with a VIP value of 2.09. Specifically, the relative fold changes of phosphoric acid and p-methylphenol were higher than 150%, while the relative fold changes of glucitol and ribose were lower than -100% (**Fig. 6b**). Compound abundance differences caused by SOM level were less than by growth stages because the absolute value of relative fold changes for the selected compounds were lower by SOM level.

Benthic N and P forms

The original concentrations of sediment TN were 3,100.62 and 5,183.74 mg kg⁻¹ dw in the low and high SOM level treatments, respectively, of which more than 95% was Org-N (**Fig. 7a, b**). The TN and Org-N showed no significant difference after the treatment period. The Inorg-N in low OM level sediment was

twice the concentration in high OM level sediment, and both increased after the treatment period (**Fig. 7c**). Among the 3 forms of Inorg-N, $\text{NH}_4\text{-N}$ was the dominant, followed by $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$. *V. natans* planting changed the Inorg-N fraction, especially in the high SOM treatment. Compared with H-N, the concentrations of $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ in the H-V treatment increased by 57% and 68.39%, respectively, whereas $\text{NO}_3\text{-N}$ decreased by 24.94%. However, no significant effects were found in the low SOM treatment (**Fig. 7d–f**).

The original concentrations of sediment TP were 1,201.62 and 1,191.55 mg kg^{-1} dw in the low and high SOM treatments (**Fig. 8a**). The ratio of Inorg-P to Org-P was 2.45 and 1.27 in low and high OM sediments, respectively (**Fig. 8b, c**). The proportions of Inorg-P and Inorg-N were higher in the low OM sediment. Fe/Al-P was the dominant Inorg-P in the low OM sediment, whereas Ca-P was dominant in the high OM sediment. *V. natans* planting changed the structure of the Inorg-P composition in the low OM sediment. Compared with L-N, the concentrations of Fe/Al-P in the L-V treatment decreased by 17.51%, whereas Ca-P increased by 16.49% (**Fig. 8d, e**). Therefore, the sediment Inorg-P was transferred from Fe/Al-P to Ca-P by *V. natans* growth. However, no significant effects were found in the high SOM treatment.

Correlation between metabolic compounds and benthic N and P forms

Pearson correlations were tested between metabolic compounds and benthic N and P forms (**Fig. 9**). Twelve compounds—lactic acid, 3-hydroxybutyric acid, phosphorous acid, sucrose, pyroglutamic acid, nonylic acid, pentadecanoic acid, proline, ribose, 2-methyl-1,2-propanediol, 3-hydroxy-decanoic acid, and adipic acid—were selected for their significant correlation with one or more sediment indicators. Lactic acid, 3-hydroxybutyric acid, and phosphorous acid were positively correlated with sediment $\text{NH}_4\text{-N}$, whereas pyroglutamic acid was negatively correlated with it. Sucrose was positively correlated with sediment $\text{NO}_3\text{-N}$, whereas pyroglutamic acid was negatively correlated with it. Nonylic acid, pentadecanoic acid, and proline were strongly positively correlated with $\text{NO}_2\text{-N}$. Sucrose and ribose were negatively correlated with TP. Finally, 3-hydroxy-decanoic acid was negatively correlated with Fe/Al-P, and adipic acid was positively correlated with OM percentage and Ca-P.

Discussion

Morphological and rhizosphere metabolic acclimatization of V. natans

Submerged macrophyte *V. natans* is a widespread species in tropical and subtropical areas globally, and is commonly used as a pioneer species in the rehabilitation of freshwater ecosystems (Zhang et al., 2016). In this study, rhizosphere metabolic profiles of *V. natans* under low and high SOM conditions were explored for their relationship with the dynamics of sediment N and P forms. Growth inhibition of *V. natans* was found in the high SOM treatment. Compared with the low SOM treatment, the biomass of roots and leaves in the high SOM treatment decreased by more than 70%. Severe growth restriction and loosened root anchorage have been found in the submerged species *Zostera marina*, *Lobelia dortmanna*, *Littorella uniflora*, *Potamogeton crispus*, and *Potamogeton perfoliatus* under the treatment of

organic enriched sediment (Mascaro et al., 2009, Raun et al., 2010). Sediment that is rich in organic matter leads to an oxygen-depleted condition, under which phytotoxins such as nitrite, sulfide, and reduced iron accumulate (Lemoine et al., 2012). Reduced sulfide oxidation capacity in anoxic sediment leads to massive sulfide invasion, which has a negative impact on plant growth and survival (Mascaro et al., 2009). Growth inhibition caused by different metabolic physiology reflects distinct rhizosphere metabolic profiles.

Primary metabolites such as organic acids, amino acids, and sugars are often found in the exudate or extracts from terrestrial and emergent plants (Van Dam and Bouwmeester, 2016). The exudation of sugars, organic acids, and amino acids is partially regulated by the permeability of the plasma membrane (Vranova et al., 2013). The strength of the rhizosphere exudation is highly species-, developmental stage-, and environment dependent. Emergent macrophyte *Phragmites australis* showed a more significant organic acid exudation than *Typha angustifolia* and *Cyperus alternifolius* in a micro-polluted constructed wetland (Wu et al., 2017). Secretion levels of sugars and sugar alcohols decreased, and amino acids and phenolics increased over *Arabidopsis* development (Chaparro et al., 2013). *Helianthus annuus* exhibited relatively higher exudation of organic acids and sugars (compounds known to affect soil nutrient bioavailability and plant–microbe associations) under low nutrient supply, as well as lower exudation of amino acids, reflecting the conservation of nitrogenous compounds (Bowsher et al., 2015). In this study, benzoic acid, glucitol, ribose, and sucrose were found to be significantly higher in the high SOM treatment (**Table S3**), under which *V. natans* growth was inhibited. These metabolites were also identified as key compounds that distinguished the metabolite profiles between low and high SOM levels. It was also found that sugars were higher in the root exudates of diluvial sand and loess loam soil type, under which the root of *Lactuca sativa* was less developed (Neumann et al., 2014). Unlike terrestrial plants, which are usually in nutrient-limited soil (Agren et al., 2012), submerged macrophytes are exposed to nutrient surplus conditions due to intensified water eutrophication. The exudation of sugars and organic acids under nutrient deficiency has been hypothesised as a mechanism for coping with environmental stress (Carvalhais et al., 2011). Increasing exudation of sugars was also found in *Zea mays* at an enhanced rate, especially for the highest nitrogen fertiliser treatment, under which plant growth was suppressed (Zhu et al., 2016). The stress condition, either nutrient-limited or nutrient surplus, stimulates the physiological response of plants to cope with the adversity through root–microbe interaction. Sugars are labile carbon sources that are easily recruited to the microbiome, and increased secretion might be an energy allocation strategy for abiotic stress response.

Changes in benthic N and P forms through rhizospheric metabolites

Distinct changes in benthic N and P forms were observed after acclimatization of *V. natans* in this study. Inorg-N accounted for less than 1% of the sediment TN, whereas Inorg-P accounted for more than 50% of the sediment TP. The Inorg-N structure in high SOM and the Inorg-P structure in low SOM were significantly changed, although the total Inorg-N and P were not significantly changed. In the H-V treatment, the concentrations of NH₄-N and NO₂-N increased by 9.73 mg/kg and 0.02 mg/kg, and NO₃-N decreased by 2.02 mg/kg. In the L-V treatment, the concentration of Ca-P increased by 108.43 mg/kg, and

Fe/Al-P decreased by 86.57 mg/kg. Sediment $\text{NH}_4\text{-N}$ was found to be positively correlated with the metabolites lactic acid, 3-hydroxybutyric acid, and phosphoric acid. The increase in $\text{NH}_4\text{-N}$ might be related to biological nitrification inhibition (BNI) by root exudates (O'Sullivan et al., 2017). The $\text{NH}_4\text{-N}$ concentration in the original high SOM was 9.58 mg/kg—almost half that in the low SOM, which might cause $\text{NH}_4\text{-N}$ deficiency for *V. natans* in the high SOM treatment. A suitable sediment $\text{NH}_4\text{-N}$ range for *V. natans* has been reported as 5–50 mg/kg (Zhu et al., 2006). Although only a few nitrification inhibition agents from root exudates have been identified, BNI activity was confirmed in different plant species, blocking either the ammonia monooxygenase (AMO) pathway or both the AMO and hydroxylamine oxidoreductase (HAO) pathways (Sun et al., 2016, Janke et al., 2018). BNI function is thought to be triggered by the synergistic effect of $\text{NH}_4\text{-N}$ presence and low pH in the root environment (Subbarao et al., 2007). The metabolites lactic acid, 3-hydroxybutyric acid, and phosphoric acid are water-soluble organic acids that are strongly ionised into the hydron. Therefore, rhizosphere metabolism of *V. natans* might promote $\text{NH}_4\text{-N}$ accumulation through anionic organic acid-induced BNI function in the high SOM treatment.

Metabolic compounds from root exudation, especially organic acids, are important drivers of the Inorg-P fractions (Zhang et al., 2009). In this study, Fe/Al-P was the dominant Inorg-P in the low SOM sediment, while Ca-P was the dominant form in the high SOM sediment. Inorg-P transferred from Fe/Al-P was transferred to Ca-P by *V. natans* growth under low SOM sediment. Inverse transformation of Ca-P to Fe/Al-P was observed in the high SOM sediment. SOM can significantly affect the earliest nucleation, subsequent particle aggregation, and transformation in soils. Organic matter, particularly humic acid, hindered Ca-P precipitation by stabilising amorphous calcium phosphate (Ge et al., 2019). *V. natans* rehabilitation could be an effective way to immobilise sediment P into Ca-P, which converts P fractions from a more labile form into a more inert form in low SOM sediment. However, this function was the opposite (and therefore not significant) in the high SOM treatment, probably due to the inhibition of SOM on Ca-P particle aggregation. Metabolites 3-hydroxy-decanoic acid and adipic acid were directly correlated with Fe/Al-P reduction and an increase in Ca-P. As opposed to studies focused on terrestrial plants, where oxalic acid, citric acid, and malic acid are usually taken as key root exudates for inorganic phosphorus transformation (Shu et al., 2007, Zhang et al., 2009), 3-hydroxy-decanoic acid and adipic acid might be emergent compounds that mediate the transformation of Inorg-P in the low SOM sediment.

Conclusions

Rhizosphere metabolic profiles of *V. natans* at low and high SOM levels were investigated for their relationship with the dynamics of sediment nitrogen and phosphorus forms. Glucitol was the key compound that differed between low and high SOM treatments. The metabolites lactic acid, 3-hydroxybutyric acid, and phosphoric acid mediated $\text{NH}_4\text{-N}$ accumulation, probably due to biological nitrification inhibition in the high SOM sediment. Additionally, 3-hydroxy-decanoic acid and adipic acid mediated the transformation of Fe/Al-P to Ca-P in the low SOM sediment, while higher SOM probably

inhibited Ca-P precipitation. This study indicated the potential risk of restoring submerged macrophytes at high SOM levels.

Declarations

Declarations: Not applicable

Funding

This study was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA23040401), the National Natural Science Foundation of China (No.51809257), the China Postdoctoral Science Foundation (No.2018M630891, 2019T120705), and the Key Research and Development Plan of Ningxia Hui Autonomous Region (No.2017BY087).

Conflicts of interest/Competing interests: the authors declare no Conflicts of interest.

Availability of data and material: not applicable

Code availability: not applicable

Authors' contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Chuan Wang, Qiaohong Zhou and Zhenbin Wu designed the experiment. Huihui Wang, Wenhao Yan, Yahua Li and Yuqing Zhao conducted the experiment. Chuan Wang, Liping Zhang and Yi Zhang analyzed the data. Chuan Wang wrote the manuscript.

Ethics approval: not applicable

Consent to participate

All the authors have approved the final version of this manuscript.

Consent for publication

All the authors agreed to submit and publish this manuscript to the journal *Plant and Soil*.

Supporting Information

Univariate analysis showing the effects of time series, SOM level, and plant, and their interactive effects on different descriptive classes, subclasses, and metabolic compounds are listed in **Tables S1–S3**. The normalised peak areas of compound subclasses that showed significant differences between the low and high SOM treatments are listed in **Fig. S1**.

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Figures

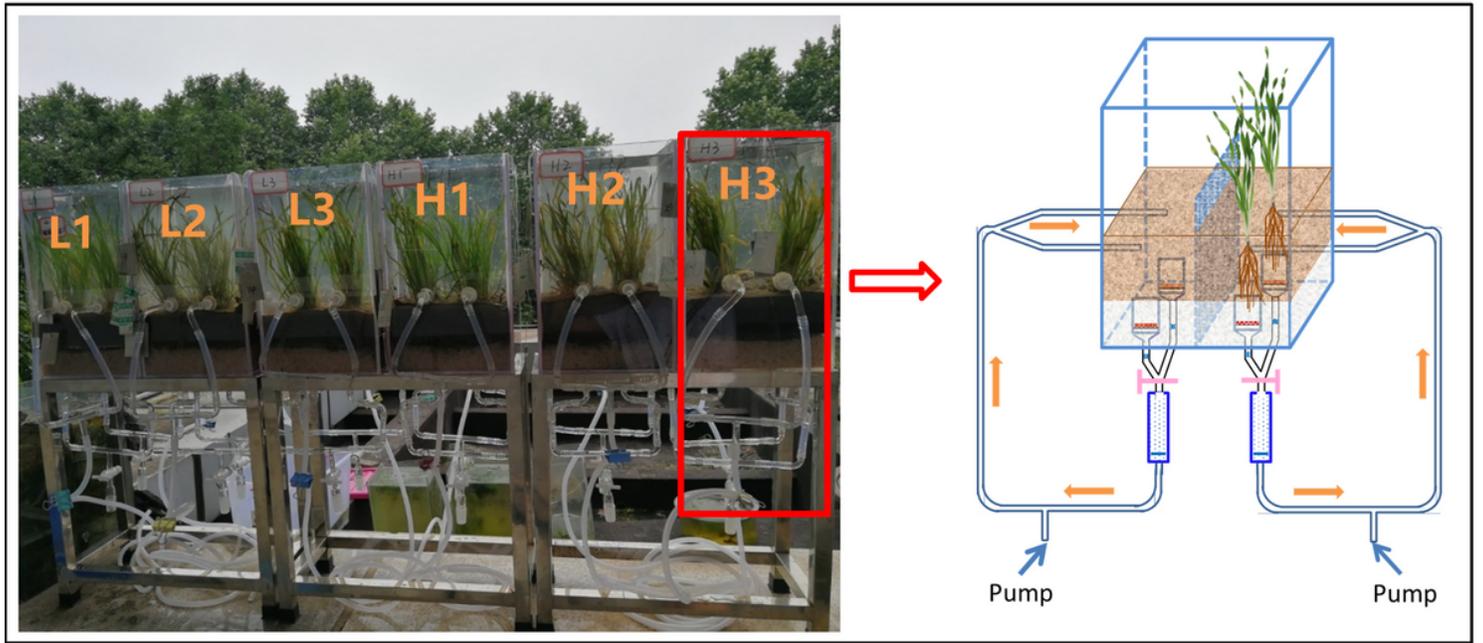


Figure 1

Field setup of rhizospheric metabolites collection system, especially for submerged macrophytes, and its schematic diagram

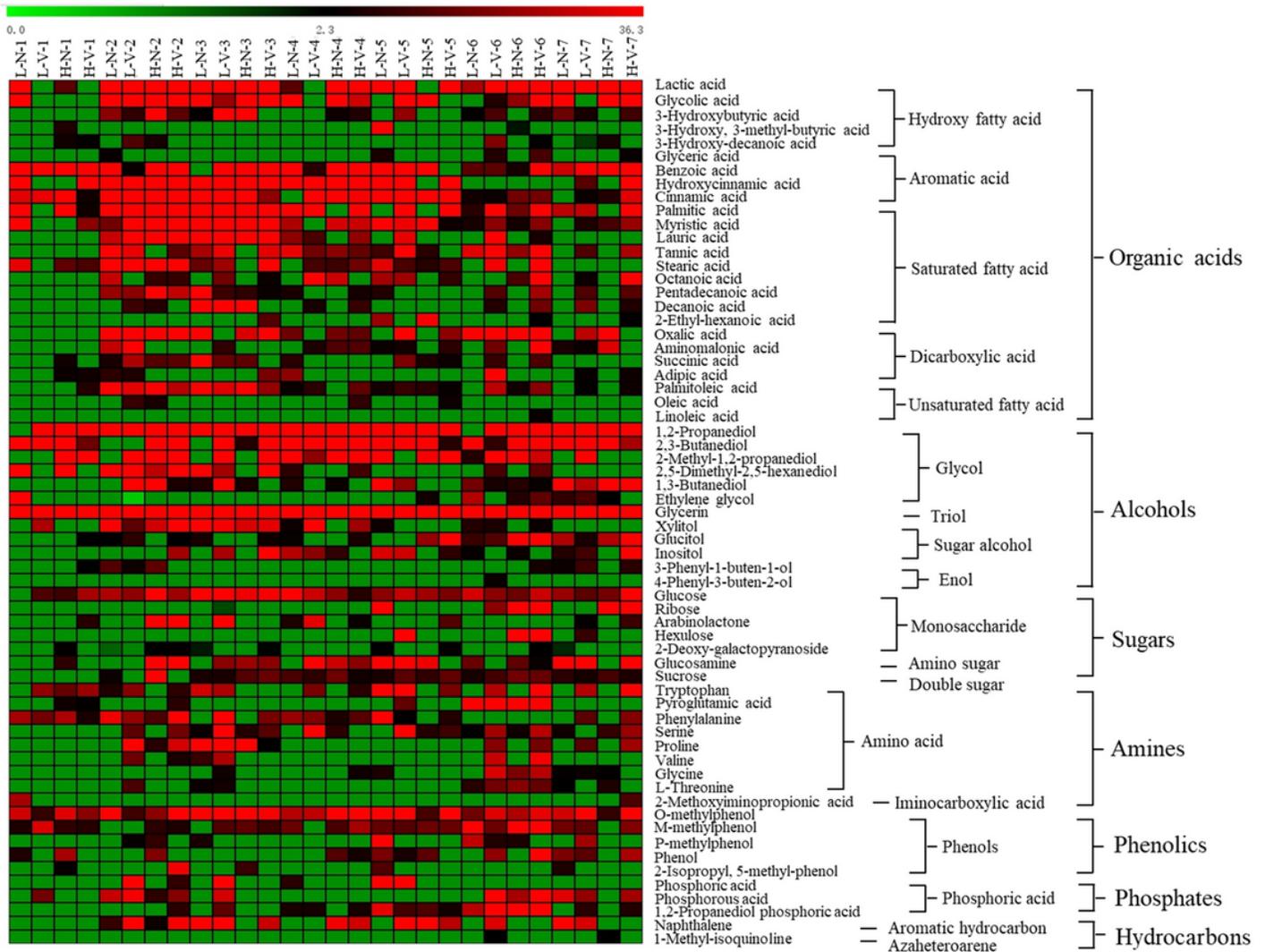


Figure 2

Paired sample t-test for the root and leaf development of *Vallisneria natans* under low and high sedimentary organic matter levels. a) Total length; and b) Total surface area. L and H represent sediment with organic matter content of 4.94% and 17.35%, respectively (as loss on ignition, LOI); and data in each column represent the mean \pm standard deviation. Significant differences between each paired sample are shown by asterisk (*, $P < 0.05$; **, $P < 0.01$).

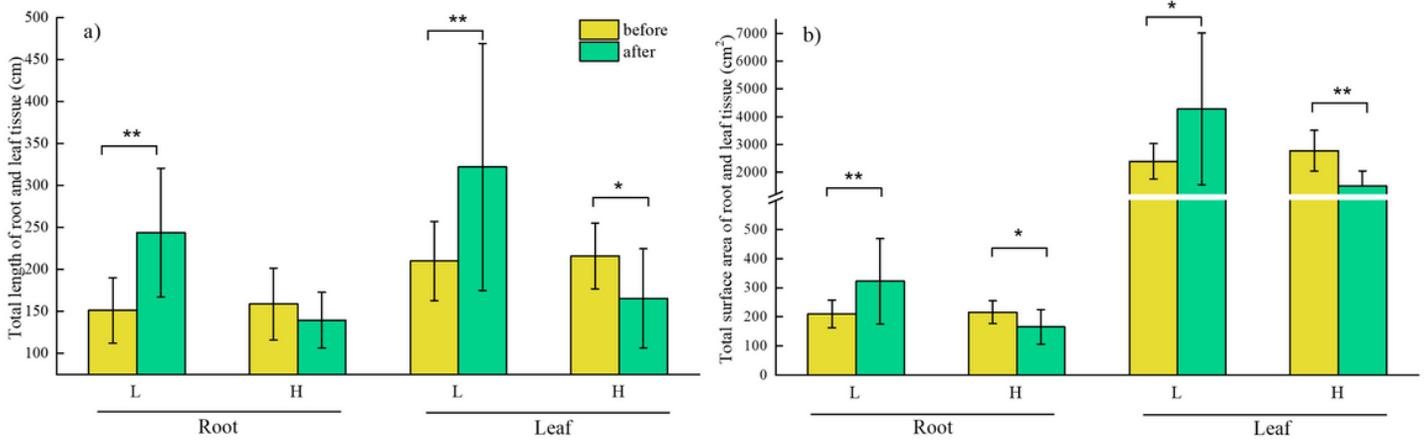


Figure 3

Heatmap of rhizospheric metabolic compounds in low and high sediment organic matter (SOM) treatments, collected for seven different incubation times. The letters V and N represent the treatment with and without *V. natans*, respectively.

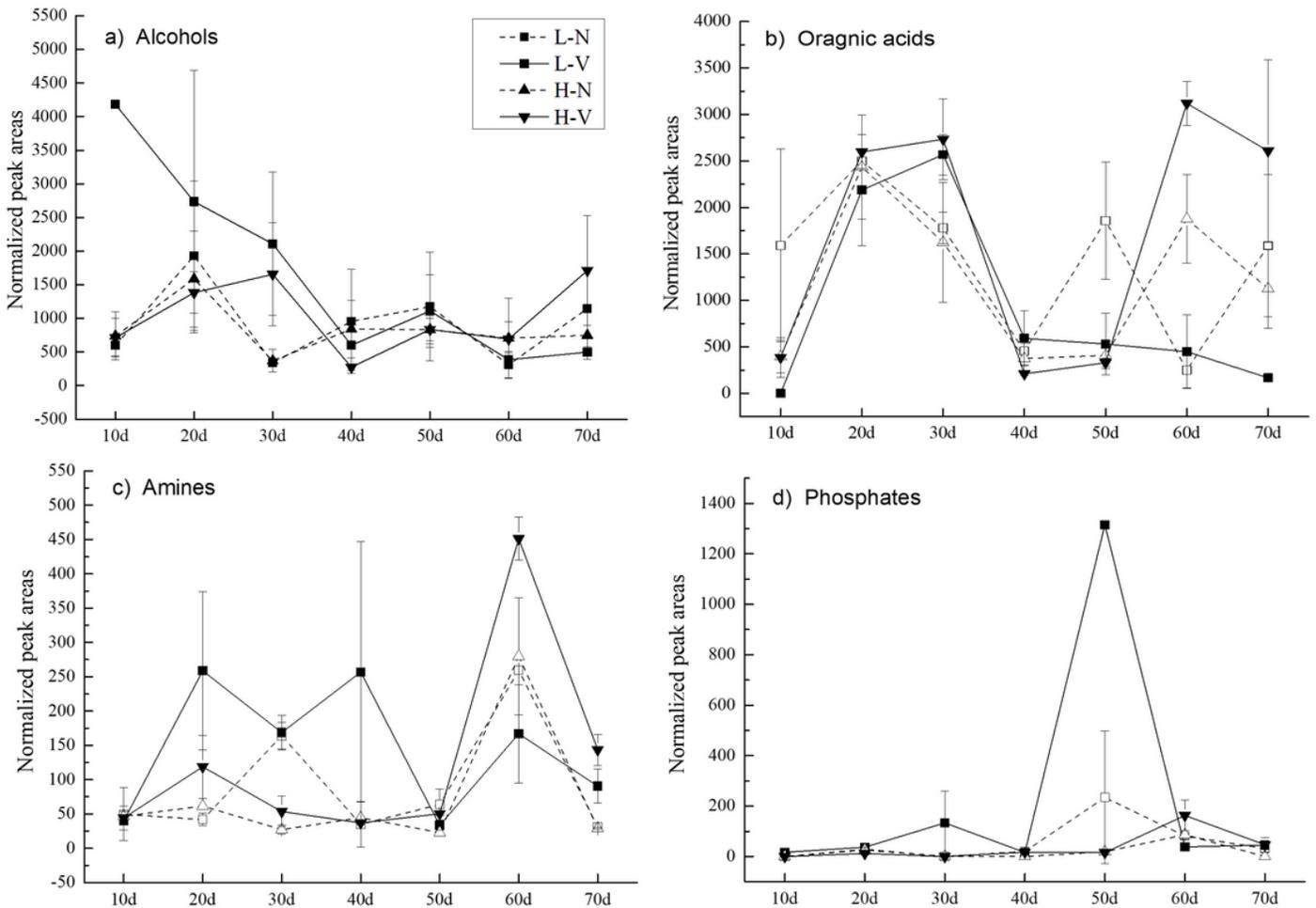


Figure 4

Normalised peak areas of compound classes showing significant differences among different time series. L and H represent low and high SOM treatment, respectively, and V and N represent treatment with and without plants, respectively.

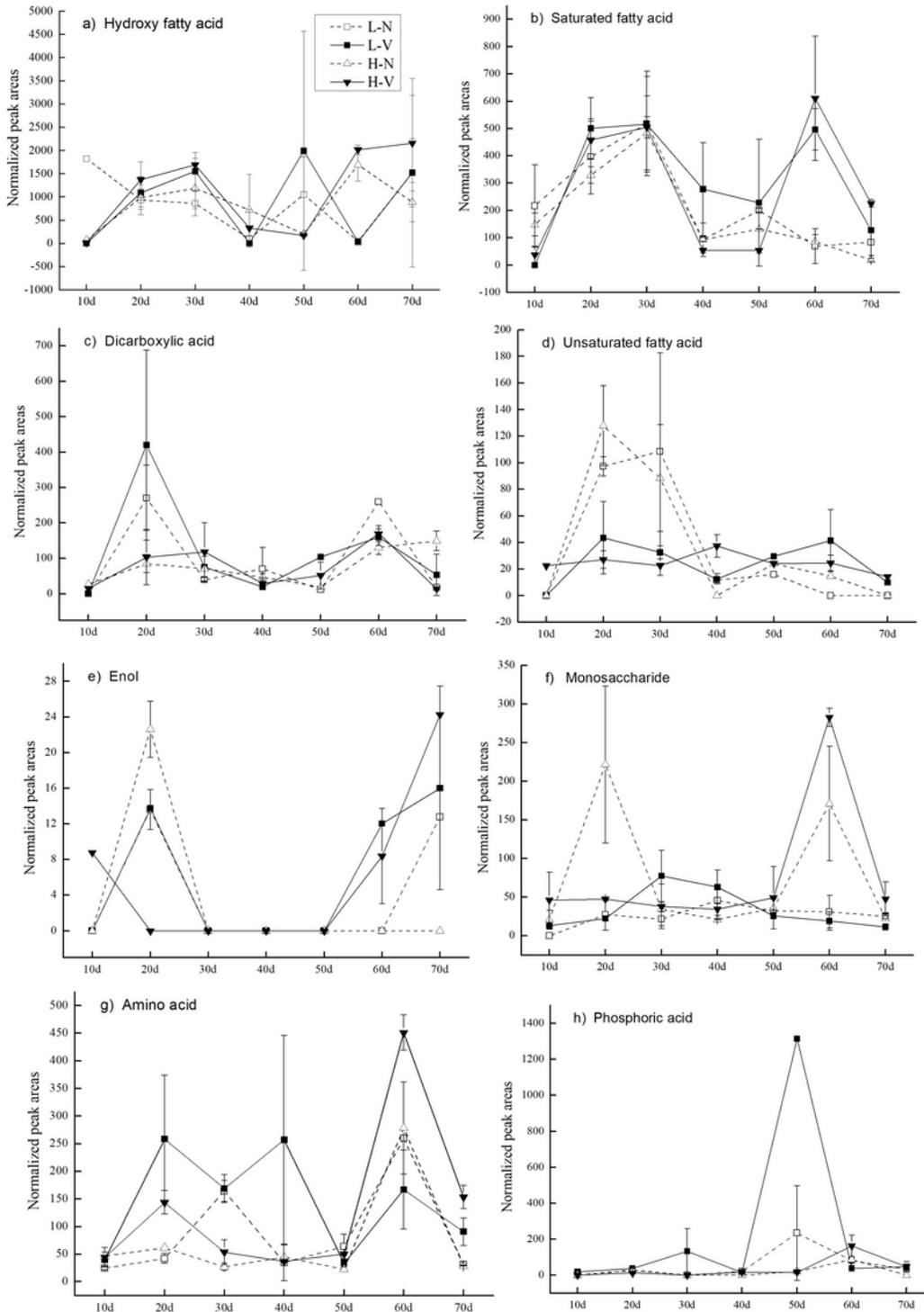


Figure 5

Normalised peak areas of compound subclasses showing significant differences among different time series. L and H represent low and high SOM treatment, respectively, and V and N represent treatment with and without *V. natans*, respectively.

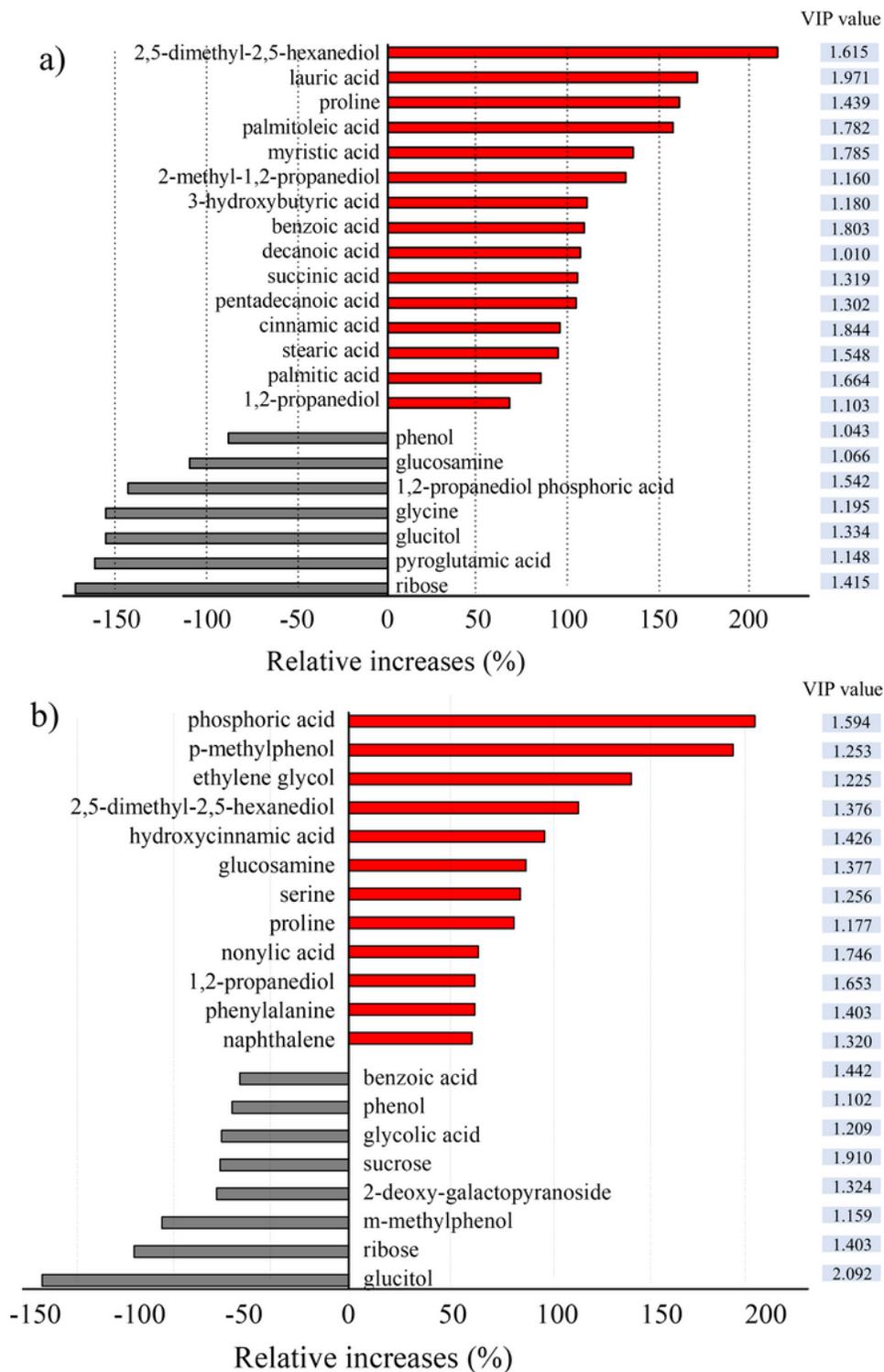


Figure 6

Relative fold changes of each compound selected by variable importance in the projection (VIP) value > 1 through partial least squares discriminant analysis (PLS-DA). a) Two-stage fold changes of metabolites,

where proportions are calculated as (Average 10-30d - Average 40-70d)/Average 10-70d; and b) High and low SOM fold changes of metabolites, where proportions in the Fig. are calculated as (Average low SOM - Average high SOM)/Average total.

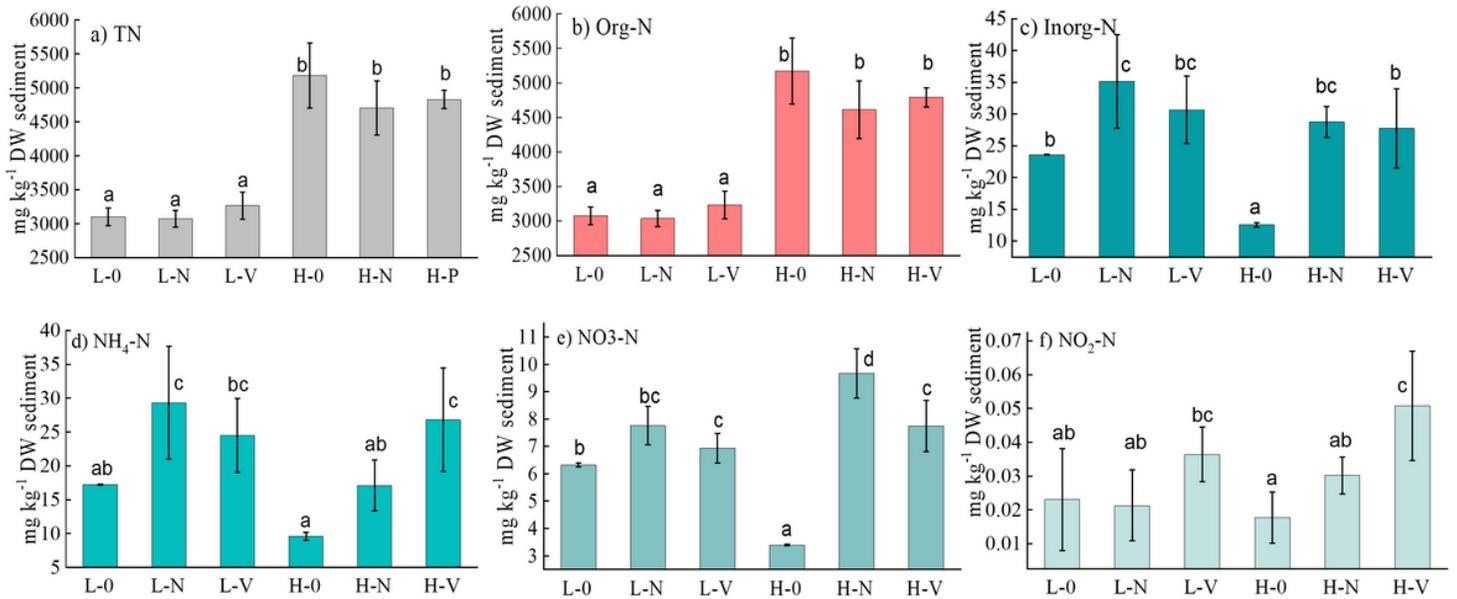


Figure 7

Different nitrogen forms in the sediment samples. O represents the original sediment status; L and H represent low and high SOM treatment; and V and N represent the sediment samples with and without V. natans after the treatment period, respectively.

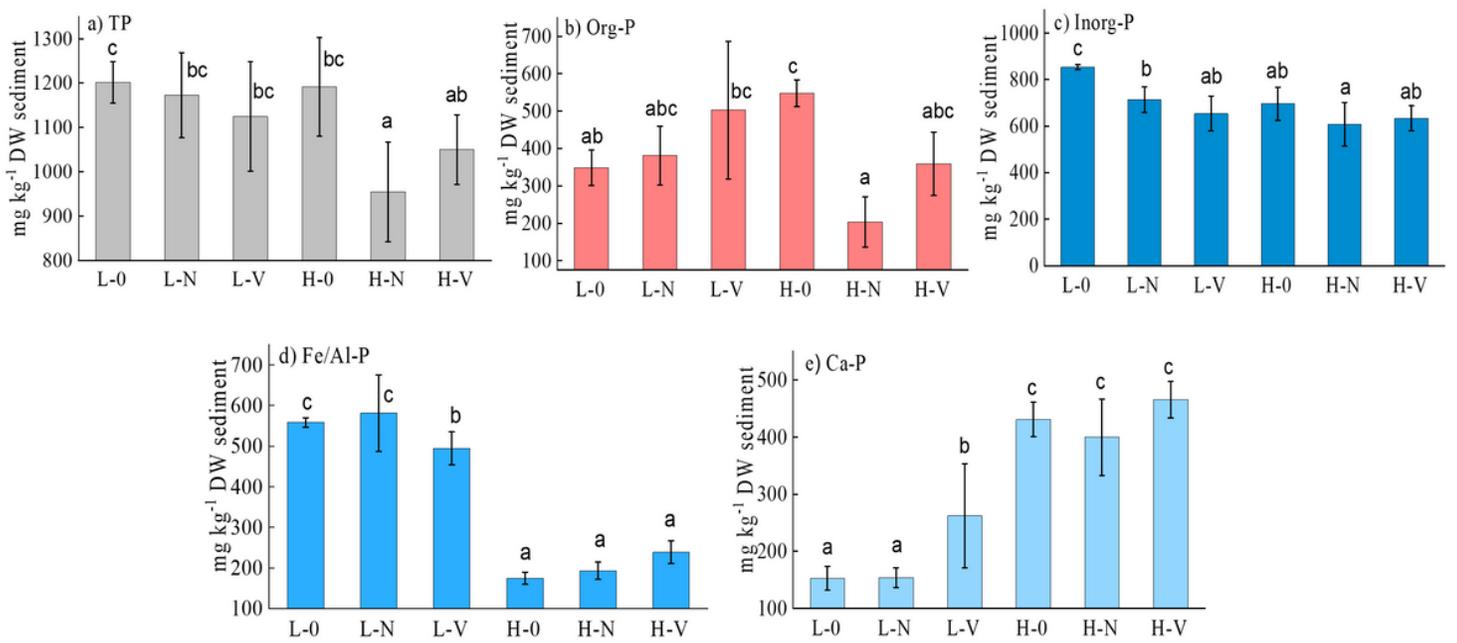


Figure 8

Different phosphorus forms in the sediment samples. O represents the original sediment status; L and H represent low and high SOM treatment; and V and N represent the sediment samples with and without V. natans after the treatment period, respectively.

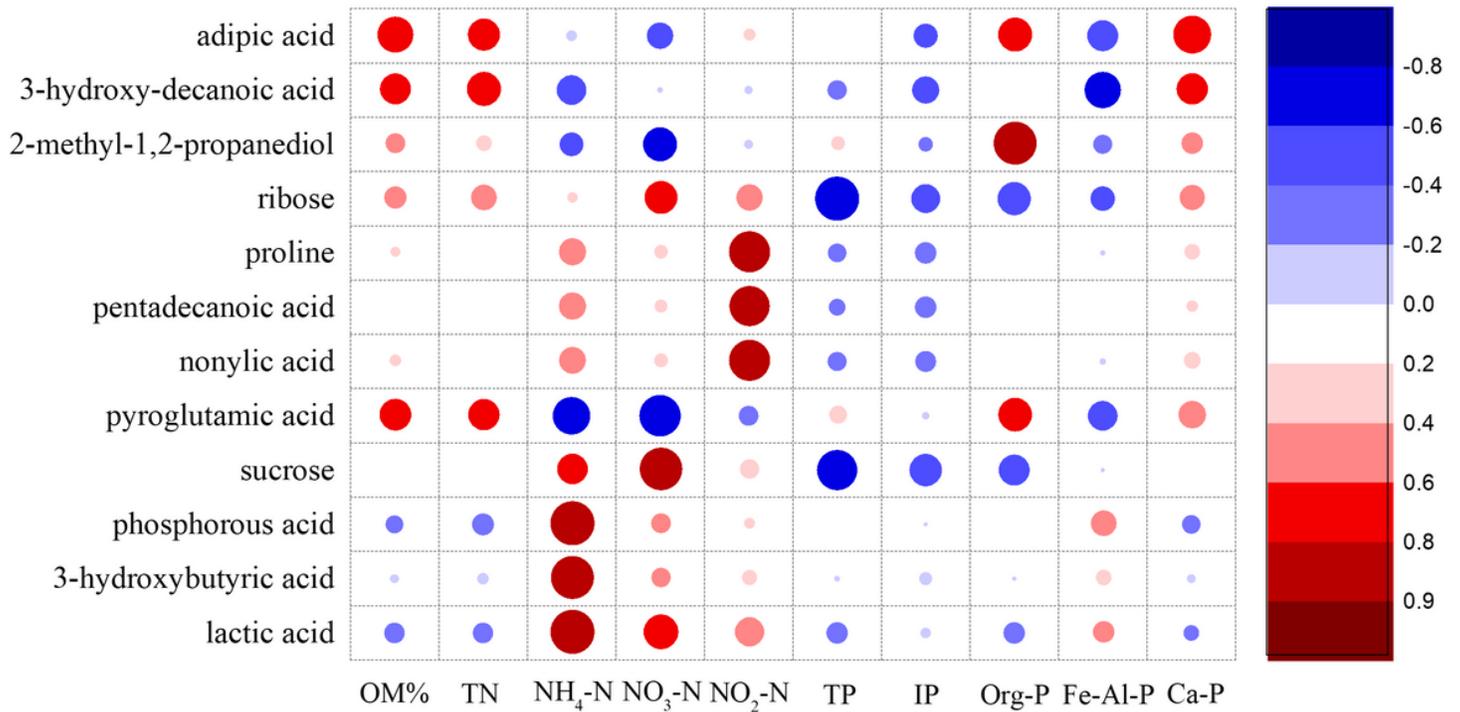


Figure 9

Pearson correlation between metabolic compounds, and benthic nitrogen and phosphorus forms.

Supplementary Files

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