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Nitrogen release and its influence on anammox bacteria during the decay of *Potamogeton crispus* with different values of initial debris biomass

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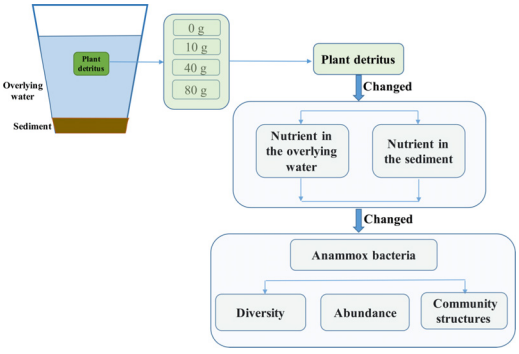
HIGHLIGHTS

- *P. crispus* was used as a model species in this study.
- The amount of mass lost and nitrogen released increased as initial biomass increased.
- DO, pH and N concentration changed obviously in the overlying water.
- O.M, pH and N concentration changed obviously in the sediment.
- The abundance, diversity and community structure of anammox bacteria varied considerably.

GRAPHICAL ABSTRACT



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ABSTRACT

Aquatic macrophytes play a significant role in the nutrient cycle of freshwater ecosystems. However, nutrients from plant debris release into both sediments and overlying water if not timely harvested. To date, minimal information is available regarding nutrient release and its subsequent influences on bacterial communities with decaying debris. In this study, *Potamogeton crispus* was used as a model plant. Debris biomass levels of 0 g (control, J-CK), 10 g dry weight (DW) (100 g DW/m^2, J-10 g), 40 g DW (400 g DW/m2, J-40 g) and 80 g DW (800 g DW/m^2, J-80 g) were used to simulate the different biomass densities of *P. crispus* in field. The physicochemical parameters of overlying water and sediment samples were analysed. The community composition of anammox bacteria in the sediment was also analysed using 16S rRNA genes as markers. The results showed that dissolved oxygen and pH dramatically decreased, whereas total nitrogen (TN) and NH_4^+-N concentrations increased in the overlying water in the initial stage of *P. crispus* decomposition. However, NO_3^--N concentration changes in the overlying water were more complicated. The concentrations of organic matter, TN and NH_4^+-N in the sediment all increased, but the rate of increase varied among the groups with different initial biomass levels, indicating that these physicochemical properties in sediment are significantly affected by debris biomass level and decay time. In addition, the order of anammox bacteria abundance was J-40 g > J-CK > J-80 g > J-10 g. Moreover, the community structure of anammox bacteria were simpler compared to that of J-CK as debris biomass level increased. The results demonstrate that *P. crispus* debris decomposition could affect the ecological distribution of anammox bacteria. Such influence clearly varies with varying amounts of *P. crispus* biomass debris. This information could be useful for the management of aquatic macrophytes in freshwater ecosystems.

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1. Introduction

Aquatic plants have great potential for removing contaminants from freshwater ecosystems (X.H. Zhou et al., 2017). However, at current, certain aquatic plants dramatically break out and spread in lakes, ponds and other bodies of water owing to enriched nutrient availability produced from rapidly expanding urban areas and agricultural and industrial activities (Smith et al., 1999; Hu et al., 2008; Garland et al., 2004). As plants litter and decay, nutrients and other pollutants in their tissues return to water columns if they are not timely harvested (Longhi et al., 2008; Liu et al., 2010; Graca et al., 2016; Zhou et al., 2018). Furthermore, external disturbances, such as animal grazing, paddling and harvesting, often produce a substantial amount of litter and fragments. Once the debris reaches water or sediment surfaces, they decompose; subsequently, the debris releases large amounts of nutrients into water columns, thus becoming a secondary pollution source (Balasubramanian et al., 2012; Li et al., 2014; Menon and Holland, 2014; Xu et al., 2014; Wu et al., 2017a,b). This secondary pollution results in the degradation of freshwater ecosystems (King and Burton, 1980; Shilla et al., 2006; Cunha-Santino et al., 2010; Y.W. Zhou et al., 2017).

Potamogeton crispus is a well-known submerged aquatic macrophyte that is widely distributed in shallow freshwater lakes, ponds, paddies, rivers and streams (Kunii, 1982; Ali et al., 2000; Qian et al., 2014; Wang et al., 2017). *P. crispus* is a submerged herbaceous perennial plant (Wang et al., 2017) that sprouts in autumn from September to October, grows throughout winter from December to February in the succeeding year, exponentially grows between March and April then dies and decays in early June (Nichols and Shaw, 1986; Wang et al., 2017; Y.W. Zhou et al., 2017). *P. crispus* can take up nutrients from both water columns and sediments; therefore, they have important implications in the nutrient cycles of freshwater systems (Flindt et al., 1999; Li et al., 2015). However, in recent years, *P. crispus* has rapidly grown, spread and covered entire lakes in a short amount of time. For example, since 2005, *P. crispus* has annually appeared in Xuanwu Lake of Nanjing, China in the winter and grows rapidly from March to April, reaching its maximum growth in early summer with a density of 30 plants/m^2 (Wang et al., 2017). In order to avoid excessive colonization, *P. crispus* has been manually harvested in the recent years (Wang et al., 2017). Such a large amount of biomass decay will inevitably have a huge negative impact on water environments. However, no information is available regarding secondary pollution owing to the decomposition of *P. crispus*. Moreover, most previous studies focused on dry mass loss and nutrient content changes in plant debris (Lee and Bukaveckas, 2002; Geurts et al., 2010; Balasubramanian et al., 2012; Hildebrandt et al., 2012; Li et al., 2013; Zhang et al., 2014; Bottino et al., 2016; Deng et al., 2016; Elmore et al., 2016; Sun et al., 2016; X.H. Zhang et al., 2017, W.Q. Zhang et al., 2017). Meanwhile the subsequent influences of plant debris decomposition on nitrogen concentration in overlying water and sediments is yet unknown. In addition, according to a field survey of the freshwater ecosystem in China, the biomass of *P. crispus* ranges from 0.4 dry weight (DW)/m^2 to 0.978 kg DW/m^2. This report leads to the question of whether or not varying amounts of debris biomass lead to varying influences.

In addition, previous studies found that anaerobic ammonium oxidation (anammox), the oxidation of ammonium through the reduction of nitrite into N_2 as the final product, is an important nitrogen removal pattern to the atmosphere under anaerobic conditions in various ecosystems (Schmid et al., 2007; Humbert et al., 2010; Zhu et al., 2011; Hu et al., 2012; Hou et al., 2013; Shen et al., 2013; Chu et al., 2015; Li et al., 2016). Previous studies confirmed that the anammox process often occurs in oxic/anoxic interface conditions (Chu et al., 2015). In such conditions, ammonium and nitrite often co-exist; therefore, mediated by anammox bacteria, they combine into molecular N under anaerobic conditions (Chu et al., 2015). Battle and Mihuc (2000) reported that plant decomposition is a complex interaction of physical, chemical, microbial and animal processes. Once aquatic plants die, the dissolved oxygen (DO) content in water depletes and sharpens, and ammonium and nitrite release into the water (Gamage and Asaeda, 2005; Wu et al., 2017a,b). Thus, we hypothesised that aquatic plant decay and decomposition could affect the ecological distribution characteristics of anammox bacteria in sediments.

Therefore, in this study, *P. crispus* was used as a model species to investigate the nutrient release from its decaying debris and its influence on anammox bacteria in sediments with different biomass levels. The specific objectives were 1) to identify the DO, pH and nitrogen concentration changes in the overlying water as *P. crispus* decays; 2) to identify the organic matter (O.M), pH and nitrogen content changes in the sediment and 3) to identify the differences in the ecological distribution characteristics of anammox bacteria due to different initial biomass levels after *P. crispus* debris has decomposed for 100 days. This information could be useful for the comprehensive evaluation of the potential influence of aquatic plant decay on sediments and overlying water in freshwater ecosystems, thereby assisting in the control of pollution caused by the decomposition of *P. crispus*.

2. Materials and methods

2.1. Sample collection and pretreatment

Plant samples of *P. crispus* were collected from the pond of Jiangsu University. The samples were washed thoroughly with deionised water to remove any impurities. Subsequently, all samples were oven dried at 65 °C to a constant weight. Afterwards, samples of the dry plant material were thoroughly mixed to obtain a homogenous starting material. Every 10 g of the dry material was separately placed into 25 cm × 25 cm nylon bags with a mesh size of 0.5 mm in order to prevent the rapid loss of small fragments of plant materials and to allow invertebrate access during the decomposition experiment (Battle and Mihuc, 2000). The sediment used in this study was collected from Jinshan Lake (32°21′34.94″N, 119°40′9.310″E), Zhenjiang, Jiangsu, China on May 31, 2014.

2.2. Experimental design

The experiment was conducted with three replicates in an indoor laboratory at room temperature from June 3 to September 12 of 2014. Plastic barrels (with volumes of 25 L and depths of 32 cm) used as experiment vessels contained approximately 3.5 cm-thick sediment. Then, 20 L deionised water was slowly poured into each experiment vessel along the wall of each barrel. Two days later, litter bags numbered 0 for control (0 g, marked J-CK), 1 (10 g, marked J-10 g), 4 (40 g, marked J-40 g) and 8 (80 g, marked J-80 g) were submerged underwater and incubated in an experiment vessel each. The numbered litter bags in the incubated system represented low (100 g DW/m^2), medium (400 g DW/m^2) and high (800 g DW/m^2) biomass densities of *P. crispus*. These densities were chosen due to the biomass range (400–978 DW g/m^2) of *P. crispus* in lakes and ponds of natural habitat ecosystems. Then, approximately 100 mL of the overlying water samples of each incubation barrel was collected at 2, 4, 8, 12, 16, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 and 100 days for nitrogen analysis. Approximately 200 g sediment samples were collected from each incubation barrel using a shovel with a handle on the 25th day (June 29, 2014), 50th day (July 24, 2014), 80th day (August 23, 2014) and 100th day (September 12, 2014) for pH, organic matter and nitrogen analysis. Meanwhile, sediments samples at 100 days were also stored at −20 °C for the molecular analyses of anammox bacteria. During the experiment, the losses in water volume due to evapotranspiration and sample collection were compensated for by the addition of deionised water up to the original level every 10 days.

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