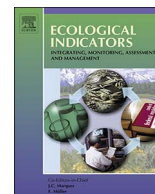




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## Original Articles

Foliar stoichiometry of carbon, nitrogen, and phosphorus in wetland sedge *Carex brevicuspis* along a small-scale elevation gradientFeng Li<sup>a,b,c,1</sup>, Jiayu Hu<sup>a,b,d,1</sup>, Yonghong Xie<sup>a,b,\*</sup>, Guishan Yang<sup>c,\*\*</sup>, Cong Hu<sup>a,b,d</sup>, Xinsheng Chen<sup>a,b</sup>, Zhengmiao Deng<sup>a,b</sup><sup>a</sup> Key Laboratory of Agro-ecological Processes in Subtropical Region, The Chinese Academy of Sciences, Hunan 410125, China<sup>b</sup> Dongting Lake Station for Wetland Ecosystem Research, Institute of Subtropical Agriculture, Changsha 410125, China<sup>c</sup> Key Laboratory of Watershed Geographic Sciences, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China<sup>d</sup> University of Chinese Academy of Sciences, Beijing 100049, China

## ARTICLE INFO

## Keyword:

Ecological stoichiometry

Dongting lake

*Carex brevicuspis*

Elevation

Inundation time

## ABSTRACT

The concentrations and ratios of plant carbon (C), nitrogen (N), and phosphorus (P) are powerful indicators of various ecological processes. The effect of elevation on the ecological stoichiometric characteristics of plants is presently unclear. Here, we examined the C:N:P ratios of the wetland sedge, *Carex brevicuspis*, along a small-scale elevation gradient and their relationships with the physicochemical characteristics of soil and inundation time of the Dongting Lake wetlands, China. The soil water content and inundation time decreased, whereas the soil bulk density increased with increasing elevation. The height, density, coverage, and aboveground biomass of plants and the organic matter content and total N and P concentrations of the soil increased initially, and then decreased with increasing elevation. The total foliar C concentration and the foliar C:N, C:P, and N:P ratios increased, whereas the total foliar N and P concentrations decreased with increasing elevation. The canonical correspondence analysis (CCA) indicated that the soil water content and inundation time were the primary factors affecting the ecological stoichiometric characteristics of *C. brevicuspis*. The total foliar C concentration and the C:N, C:P, and N:P ratios decreased, but the total foliar N and P concentrations increased with increasing soil water content and inundation time. Our findings highlight the effects of elevation on plant growth and stoichiometric characteristics, which are applicable to the conservation and management of the wetlands dominated with *C. brevicuspis*.

## 1. Introduction

Ecological stoichiometry mainly focuses on the mass balance of multiple nutrient elements in the ecological systems (Cross et al., 2005; Rong et al., 2015). Plant nitrogen (N) and phosphorus (P) are the most important nutrient elements and the foundation of chemistry composition of living organisms on earth (Elser et al., 2000; Mao et al., 2016). The stoichiometry and relative abundances of carbon (C), N, and P in plants are the powerful indicators of the diverse ecological processes such as population stability, competition, community organization, nutrient limitation, food web, and decomposition (Elser et al., 2000; Güsewell et al., 2003; Yu et al., 2012). Therefore, the studies on plant ecological stoichiometry would enhance our understanding of the growth and nutrient-use strategies of plants, as well as their responses to various environmental stresses.

Nutrient stoichiometry is affected by environmental factors as well as plant physiological processes (Liu et al., 2015). Variations in plant stoichiometry have been documented at different scales, from the molecular and organismal level to the global-scale level (Elser et al., 1996; He et al., 2006; Xia et al., 2014) in different plant functional groups (Wang and Moore, 2014; Zhang et al., 2015), different plant organs of the same species (Li et al., 2014), and in plants growing under various environmental factors such as nutrient, water depth, and light (Cronin and Lodge, 2003; Li et al., 2013a; Xing et al., 2013; Mao et al., 2016).

In freshwater wetlands, the water level plays the most important role in determining plant growth and community structures, and is closely related to the distributing elevations of plants (Deng et al., 2013; Li et al., 2013b). At different distributing elevations in some floodplains and river-connected lakes, a substantial change has been noticed in the soil water content and flooding duration and frequency,

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Received 29 August 2016; Received in revised form 10 March 2017; Accepted 19 April 2017

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which in turn, affect the aeration, physical structure, and nutrient availability of soil (Dwire et al., 2006; Anderson and Lockaby, 2011; Deng et al., 2013). Previous studies have extensively investigated the influences of water level on the growth performance, reproduction, and competition in plants (Deegan et al., 2007; Deng et al., 2013; Li et al., 2015). Moreover, a few studies conducted on the forest ecosystems have confirmed that the foliar N and P concentrations can increase, decrease, or remain unchanged along an elevation gradient (Du et al., 2016), while the leaf N:P ratio decreased with increasing elevation (Fisher et al., 2013). However, limited studies have been conducted to investigate the changes in the ecological stoichiometry of plants along an elevation gradient in wetland ecosystems.

Dongting Lake, located in the middle reach of the Yangtze River, is the second largest freshwater and a typical river-connected lake in China (Xie and Chen, 2008). In this lake, *Carex brevicuspis* is one of the dominant sedge species, and is distributed widely at different elevations with various important ecological functions, such as serving as the main food resource for migratory birds and a spawning ground for migratory fish. However, in the recent years, the *C. brevicuspis* community has been seriously degraded because of the changes in hydrological regime caused by intensive anthropogenic disturbances. Therefore, it is an ideal species for investigating the relationship between elevation gradient and plant stoichiometry.

In the present study, we focused on the foliar stoichiometric characteristics (including the total C, N, and P concentrations and the C:N, C:P, and N:P ratios) of the *C. brevicuspis* community along a small-scale elevation gradient in Dongting Lake. We determined the physicochemical characteristics of soil (water content, bulk density, and total N, total P, and organic C concentrations) and inundation time at different elevations. The aims of this study were to examine (1) the difference in growth and foliar stoichiometric characteristics of *C. brevicuspis* at different elevations, and (2) the relationships between plant foliar stoichiometric characteristics and soil physicochemical characteristics and inundation time.

## 2. Materials and methods

### 2.1. Study site

Dongting Lake (28°30′–30°20′N, 111°40′–113°10′E) receives inflow mainly from the four rivers (Xiang River, Zi River, Yuan River and Li River) and the four channels (Songzi, Taiping, Ouchi and Tiaoxian) linked to the Yangtze River (Fig. 1). The water level changes significantly with different seasons; the wetlands are usually flooded during May–October and relatively dry during November–April. The mean annual temperature is 16.4–17.0 °C and the average annual precipitation is approximately 1382 mm (Chen et al., 2014).

This study was conducted on the Dingzidi lakeshore (29°25′25.8″N, 112°56′45.8″E) located in East Dongting Lake. *Carex brevicuspis* is distributed widely in this lakeshore from the adjacent areas of the water body to the embankment. On this lakeshore, *C. brevicuspis* forms mono-dominant communities along a mild slope of 5–10°. The elevation ranged from 22.6 to 26.2 m on this lakeshore, making it an ideal site for investigating the stoichiometric characteristics of *C. brevicuspis* at different elevations.

### 2.2. Study species

*Carex brevicuspis* is widely distributed in Taiwan and eastern mainland China (Dai et al., 2010). It can form mono-dominant communities or exist co-dominantly with other species, such as *Miscanthus sacchariflorus* and *Polygonum hydropiper*, in Dongting Lake. Depending upon the flooding pattern of Dongting Lake, *C. brevicuspis* usually has two growing seasons; it flowers and fruits in April or May before the flooding begins, and is completely submerged during the flooding season. After flooding, the shoots emerge immediately (November) and

keep growing till January. In January, the above-ground plant parts become withered because of the cold temperature. Subsequently, the new ramets emerge and grow rapidly in February or March (Deng et al., 2013).

### 2.3. Field sampling

In May 2012, the sampling sites were established on the Dingzidi lakeshore before flooding. Seven transects, parallel to the water body and 150 m away from each other, were set up. The first transect was approximately 20 m away from the water body. Nine plots of 1-m<sup>2</sup> area were established in each transect, and the distance between each plot was 50 m. The density, height, and coverage of *C. brevicuspis* shoots were recorded. Plant density was defined as the number of plants in each plot. Plant height was measured using a steel tape with 0.1-cm scale. Additionally, a global positioning system (MG758E; Beijing UniStrong Science & Technology Co., Ltd., Beijing, China) was used to record the geographical coordinates of plants. Because *C. brevicuspis* is a perennial acaulescent herb, it is difficult to distinguish between the dead live roots. Therefore, we chose the leaves of *C. brevicuspis* as our study material. The leaves of the mature plants with similar growth performance were collected from each plot. Mature plant leaves were defined based on the length of leaves. Subsequently, all aboveground parts of the plants were mowed, and stored in the labeled polyethylene bags for measuring their aboveground biomass later.

After examining the vegetation, a 100-cm<sup>3</sup> soil sampler was used to collect the soil samples for measuring the soil bulk density in each plot of 0–20 cm depth. Five such soil samples were collected from each of the four corners and the center of each plot. Subsequently, these five samples were thoroughly mixed into one composite sample for each plot. All plants and soil samples were placed in the labeled polyethylene bags and transported to the laboratory, where they were kept at 4 °C until analysis. The samples were analyzed within less than 20 days.

### 2.4. Laboratory analysis

All leaf samples were oven-dried to a constant weight at 70 °C, and subsequently ground for further analysis. Total N and C concentrations of leaves were measured by an elemental analyzer (Vario MAX CN, Elementar, Germany), and total P concentration of leaves was measured by molybdenum blue colorimetric method after digesting the leaf samples in a solution of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Zhang et al., 2015).

The soil samples were air-dried and sieved to remove the coarse fragments (< 0.15 mm for total N, total P and organic C contents; < 0.25 mm for other index analyses). The soil organic C content was measured by the wet oxidation of organic matter with a solution of potassium dichromate (KCr<sub>2</sub>O<sub>7</sub>) and H<sub>2</sub>SO<sub>4</sub>, followed by the back titration with ferrous sulfate (FeSO<sub>4</sub>; Zhang et al., 2015). The samples were then analyzed for the total soil N concentration using Kjeldahl method and for the total soil P concentration using acid digestion with a solution of H<sub>2</sub>SO<sub>4</sub> and perchloric acid (HClO<sub>4</sub>) solution (Zhang et al., 2015). The soil water content was measured by oven-drying method (Zhang et al., 2015). Additionally, using the digital elevation model (1:10,000) of Dongting Lake (Changjiang Water Resources Commission, Ministry of Water Resources, China, 1995) and the coordinates of each plot, their distributing elevations were calculated with an accuracy of 0.1 m. The inundation time was calculated on the basis of the daily water level data (8:00 a.m.) obtained from Chenglingji hydrological gauging station in 2011.

### 2.5. Data analysis

One-way analysis of variance (ANOVA) was performed in conjunction with Duncan's test to determine the effects of elevation on the stoichiometric characteristics of *C. brevicuspis*. Tukey's test was used for

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