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Spatial Distribution and Environmental Determinants of Denitrification Enzyme Activity in Reed-Dominated Raised Fields

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Abstract: Denitrification is an important process of nitrogen removal in lake ecosystems. However, the importance of denitrification across the entire soil-depth gradients including subsurface layers remains poorly understood. This study aims to determine the spatial pattern of soil denitrification enzyme activity (DEA) and its environmental determinants across the entire soil depth gradients in the raised fields in Baiyang Lake, North China. In two different zones of the raised fields (i.e., water boundary vs. main body of the raised fields), the soil samples from −1.0 m to 1.1 m depth were collected, and the DEA and following environmental determinants were quantified: soil moisture, pH, total nitrogen (TN), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total organic carbon (TOC), and rhizome biomass of *Phragmites australis*. The results showed that the soil DEA and environmental factors had a striking zonal distribution across the entire soil depth gradients. The soil DEA reached two peak values in the upper and middle soil layers, indicating that denitrification are important in both topsoil and subsurface of the raised fields. The correlation analysis showed that the DEA is negatively correlated with the soil depth ($p < 0.05$). However, this phenomenon did not occur in the distance to the water edge, except in the upper layers (from 0.2 m to 0.7 m) of the boundary zone of the raised fields. In the main body of the raised fields, the DEA level remained high; however, it showed no significant relationship with the distance to the water edge. The linear regression analysis showed significant positive correlation of the DEA with the soil TN, NO_3^--N, NH_4^+-N, and TOC; whereas it showed negative correlation with soil pH. No significant correlations with soil moisture and temperature were observed. A positive correlation was also found between the DEA and rhizome biomass of *P. australis*.

Keywords: denitrification enzyme activity (DEA); nitrate removal; raised fields; Baiyang Lake; rhizomes

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1 Introduction

Denitrification is a key process that affects the nitrogen cycling in wetland ecosystems (Burgin *et al*., 2010; Akatsuka and Mitamura, 2011). Wetlands have been widely acknowledged as the important regions to improve the water quality through plant uptake and microbial immobilization of excess nutrients via soil denitrification. The nitrogen amount reduced by plant uptake and microbial immobilization is returned to soils when the environmental conditions are changed (Liu *et al*., 2011). Microbially-mediated denitrification is the main process to remove nitrogen permanently in significant amounts from the wetlands (Howarth *et al*., 1996; Li *et al*., 2011). Moreover, microbial denitrification is an anaerobic process and converts nitrate to N_2O and N_2, which are then released into the atmosphere (Khalil and Richards, 2011). The reduction of nitrate to nitrous ox-

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ide (N_2O) and nitrogen (N_2) is performed by the denitrifying bacteria in soils, subsoils, and water bodies (Song *et al.*, 2011). Denitrification is difficult to quantify directly, and the measurement of soil enzyme activity, the biological indicator for microbial community functions, helps to better understand the transformation and the fate of nitrate (Khalil and Richards, 2011).

Much work has been devoted to studying how denitrification varies as a function of soil characteristics, vegetation factors, and water quality. Sites with different environmental conditions exhibit different rates of denitrification; however, because the environmental factors often interact dynamically, it is somehow difficult to identify the main factor responsible for denitrification (Bastviken *et al.*, 2005; Stevenson *et al.*, 2011). Because biological denitrification requires a source of nitrate/nitrite, organic carbon, and anoxic conditions, the soil and water characteristics directly influence the denitrification process (García-Ruiz *et al.*, 1998). The results of most studies showed that the carbon availability and soil temperature significantly influence the denitrification efficiency of the soils (Burford and Bremner, 1975; Peterson *et al.*, 2013; Reisinger *et al.*, 2013). However, the influence of soil moisture and nitrate nitrogen (NO_3-N) on denitrification efficiency varies in different research fields (Griffiths *et al.*, 1998). Moreover, the vegetation characteristics significantly affect the physicochemical characteristics of water and soils, thus exhibiting indirect influence on biological denitrification (Racchetti *et al.*, 2011; Li *et al.*, 2012). Plant residues enrich the soil nutrients, and the highest denitrification efficiency is generally found in the topsoil. Some studies found no significant differences in the topsoil denitrification enzyme activity (DEA) under different vegetation covers (Priha *et al.*, 1999; Dhondt *et al.*, 2004), while others found that plant biomass and vegetation significantly positively affect the topsoil DEA (Liu *et al.*, 2011). Plant root growth has a significant influence on the DEA in the deep soil layers (Dhondt *et al.*, 2004).

Despite widespread regarding the denitrification process in natural wetlands, few studies have explored the spatial distribution and environmental determinants of DEA across the entire soil depth gradients in the human-modified wetland systems. Human intervention leads to the changes of the water and soil characteristics of wetlands, and the DEA is changed along with it. Raised field system is a traditional human-modified agricultural system with a long history of agricultural production in Asia and the Americas (Erickson and Candler, 1989; Wilson *et al.*, 2002; Bandy, 2005). The raised fields consist of a series of elevated planting platforms surrounded by excavated ditches, and are constructed in the seasonally flooded plains in widespread parts of lowland and highland (Denevan, 1970; Turner, 1974). Crops are grown on the platforms surrounded by ditches connected to inlet and outlet canals. The platforms can be further divided into two parts by their structural and functional features: the transitional boundary zone and main body. In recent years, it is increasingly recognized that the raised fields play a direct and important role in removing nutrients, particularly nitrogen loads from agricultural runoff and domestic sewage (Mitsch, 1992; Carney *et al.*, 1993). The boundary zone is the interface between the terrestrial and aquatic ecosystems, which often contain hot zones for biogeochemical cycles (Zhu *et al.*, 2013). However, the biological process in the main body of raised fields is not yet fully understood.

With varying environmental conditions in space, the spatial variations and environmental factors for the soil DEA is important for identifying regions where the pollutant load can be effectively decreased, thus helping in the design of raised fields. The objectives of this study are as follows: 1) to identify the spatial distribution of DEA across the entire soil depth gradients, including subsurface layers in the raised fields of Baiyang Lake and 2) to correlate the DEA with soil and plant properties affecting biological denitrification.

2 Materials and Methods

2.1 Study site

Baiyang Lake (38°43′–39°02′N, 115°38′–116°07′E) is the largest freshwater inland lake in the North China Plain. It covers an area of approximately 366 km^2 and has a temperate continental monsoon climate with an average annual precipitation of 510 mm and evaporation of 1690 mm. The elevation range is 5.5–6.5 m and the whole lakebed is generally high in the west and low in the east, with a water capacity of 1.07×10^{10} m^3. Over the last several decades, due to various pollution sources from industrial and agricultural production, the Baiyang Lake was in a eutrophic state. The construction of upstream dams and reservoirs resulted in lowering water level and deterioration of ecosystem (Cui *et al*., 2010).

There is approximately 9400 ha of raised fields with more than 3700 ditches in the Baiyang Lake, dividing the whole body of water into 140 smaller shallow lakes. Raised fields in the Baiyang Lake were mainly built during 1965–1966, in order to respond to the Government's requirement to 'grow crops in swamp' (CCLCAC, 2000). Raised fields cover approximately 22% of the total area of the lake and consist of dug ditches and elevated platforms (Fig. 1). *Phragmites australis* is the dominant aquatic macrophyte growing in raised fields. Platforms are higher than the surface of the lake and surrounded by ditches, where the water level can be controlled or fluctuate hydrologically. The height from the ditch bottom to the platform surface ranges from 2 m to 4 m. The widths and lengths of the platforms also vary, roughly from 30–50 m and 100–300 m, respectively, according to the site topography and landforms, and the average ditch width is between 2 m and 8 m in the lake.

2.2 Sampling and analysis

One of the well-managed raised fields at Duancun, Anxin County, Hebei Province was chosen for sampling in August 2011 (Fig. 1). The junction of the raised fields and the lake surface was set as 0 m to measure the horizontal distance to the water edge. Obvious changes occurred in the plant height and the coverage of *P. australis* at 5 m from the water edge in previous surveys, so 5 m was defined as a dividing point between the transitional boundary zone and the main body of raised fields. There were 21 soil profiles, being collected at intervals of 0.5 m within the initial 5 m and at intervals of 10 m from 10 m to 100 m. The outermost edge has the denser sample profiles, which is demonstrated with spots marked with blue dots in Fig. 2. The water level was also set to 0 m in a vertical direction. Various soil samples were collected from topsoil to 1.0 m below the water level, and divided into sub-samples at 10 cm intervals. The topsoil was 1.1 m above the water level in sampling. The spatial variability of denitrification enzyme

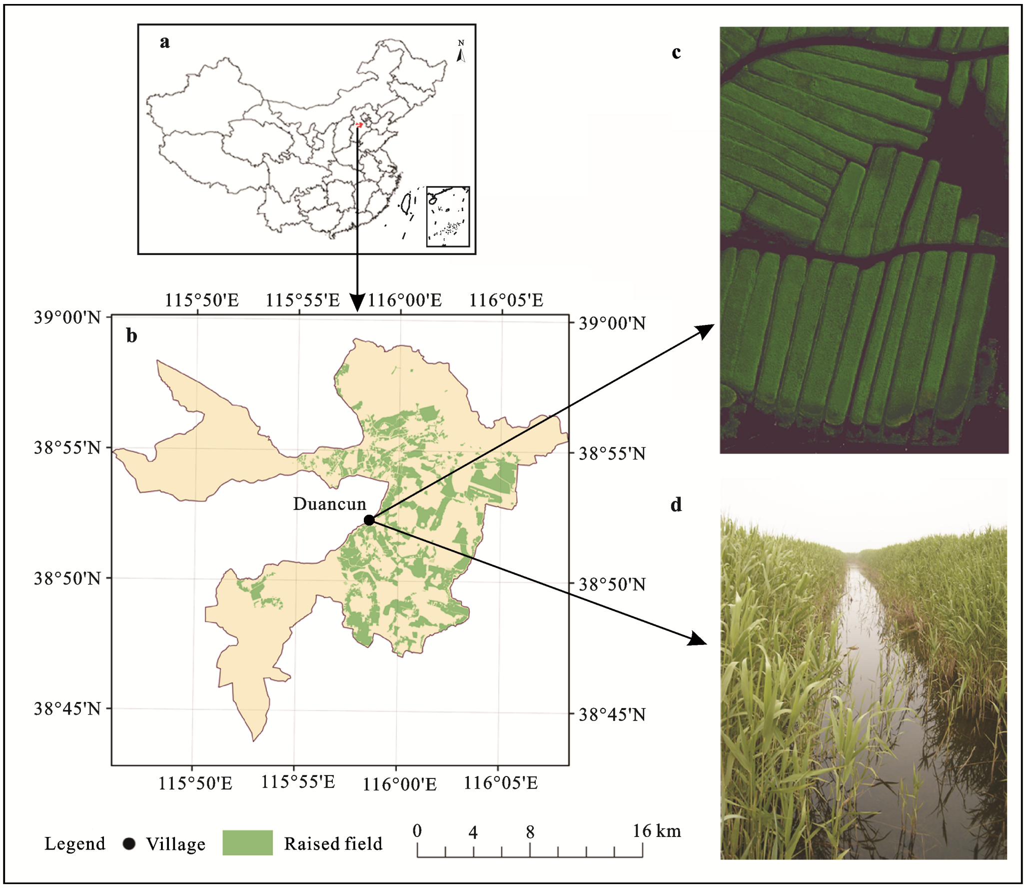

Fig. 1 Spatial distribution and landscape of raised field systems. a, geographical location of Baiyang Lake in China; b, spatial distribution of raised fields in Baiyang Lake; c, remote sensing of raised fields with Quick Bird satellite imagery; d, side elevation of raised fields

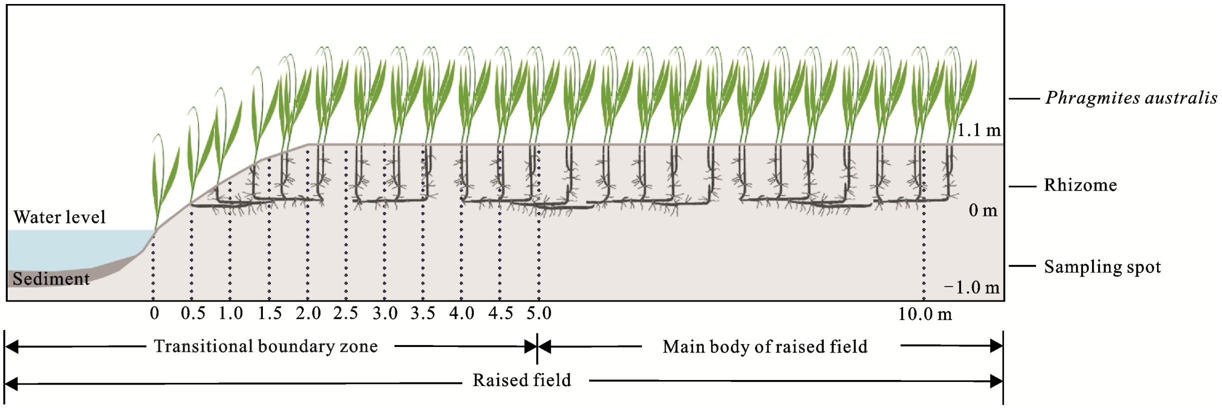

Fig. 2 Distribution of sampling spots in sampling raised fields

activity and soil nutrients were investigated for each soil sample.

P. australis rhizome litters were the major carbon and nitrogen sources for raised fields, due to harvesting of the high amount of aboveground material at the end of November every year. Rhizome biomass was selected as a marker to reflect the growth status of *P. australis*. Rhizomes of *P. australis* were taken carefully under a surface area of 0.25 m^2, to a constant depth of 10 cm using a small, metallic rake. Soil cores were collected using a soil auger (Eijkelkamp, Netherlands, 01.11.SO) and cut into 10 cm segments. Soil (10 cm in depth) temperature was monitored using WET sensor kit (Delta-T Devices, UK, Model WET-2), as soon as possible. A water pump was used to draw out water from the quadrates, in time to make rhizome samples collection easier. Triplicates were used for each sample to ensure the precision of all the data.

All the soil and plant samples were stored in sealed polyethylene bags, and transported in a cooler to the laboratory. The 100-g soil of each sample was oven dried at 105℃ for 24 h, and weighed for moisture content determination. The remaining soil of each sample was freeze-dried and sieved through a 2-mm sieve to remove coarse debris and grass roots. Then, half of the sieved soil samples were ground to a fine powder (100 mesh) and used to determine the total nitrogen (TN) in soil. The TN content in powdered soil was determined using an Elemental Analyzer (Elementar, Germany, Vario EL cube). Nitrate-nitrogen (NO_3-N) and ammonia-nitrogen (NH_4-N) contents in these soil samples were determined using auto analyzer (Bran+Luebbe AA3, Germany, Auto-analyzer 3). Total organic carbon (TOC) in the soil samples was measured using a high sensitivity TOC analyzer (Shimadzu, Japan, TOC-V_{CPH}). The pH values of soil samples (soil ∶ water = 1 ∶ 5) were measured using a pH meter (Hach, USA, A330113), and soil particle analyses were performed using a particle size analyzer (Microtrac, USA, S3500). Rhizomes of *P. australis* were cleaned from soil samples using a pressurized water spray, and then subsequently dried in oven at 60℃ to a constant weight for biomass determination.

Measurements of DEA, proposed by Smith and Tiedje (1979), have been widely used to evaluate the activities of existing denitrifying enzymes. The 5 g of soil was first placed in a 100-mL sterile, airtight, Hungate-type anaerobic culture tube. Then, a solution containing 0.18 g/L glucose, 0.1 g/L KNO_3, and 1 g/L chloramphenicol was added to each tube. The tubes were flushed with nitrogen (N_2) to remove Oxygen (O_2) and amended with Ethyne (C_2H_2) to a concentration of 0.05 atm, which provide anaerobic conditions and inhibition of Nitrou oxide (N_2O)-reductase activity. The soil samples were shaken at 25℃ for 6 h, and then the N_2O efflux was measured using a gas chromatograph with an electron capture detector (Agilent, USA, 7890A).

2.3 Data analysis

The soil properties, rhizome biomass of *P. australis*, and DEA data were collected to generate the spatial distribution maps using Kriging module of ArcGIS software. Pearson correlation coefficients were used to perform the relationships between soil position and soil characteristics, soil position and plant. Then, the results were analyzed by linear regression analysis to reveal the effects of environmental factors on DEA. All statistical analyses were performed using the software package, Origin 8.0 and SPSS 17.0.

3 Results

3.1 Spatial distribution of soil properties and *P. australis* rhizomes across soil profiles

The physicochemical properties of the soil samples are listed in Table 1. The soil pH showed alkaline conditions and narrow pH ranges. The soil temperature and moisture varied from 22.3℃ to 33.4℃ and from 18.62% to 42.61%, respectively. The concentrations of TN, NH_4-N, NO_3-N, and TOC varied significantly owing to different depth and distance to the edge of the lake. The maximum concentrations of soil nutrient were 1.03–78.67 times higher than the minimum concentrations. The largest and smallest differences in the physicochemical properties were observed for the NO_3-N and pH. The soil samples were dominated by high silt (36.55%–68.21%) and sand (22.13%–50.76%) contents.

Some preferred soil properties, which strongly control the denitrification process were selected to analyze their spatial distribution and relationships with the DEA based on the literature studies (Dhondt *et al.*, 2004; Cao *et al.*, 2008; Hill *et al.*, 2000; Li *et al.*, 2011; Al Ghadban *et al.*, 2012; Batson *et al.*, 2012; Pan *et al.*, 2012; Yu *et al.*, 2012). Significant differences in soil properties were found in the soil samples (Fig. 3). The horizontal distribution patterns of soil characteristics showed slight differences, while the vertical distribution was a zonation pattern. The high temperature layer appeared in the top and bottom layers. The moisture content within the initial 1.0 m from the water edge and in the depth of −0.1 m to −0.5 m showed the highest content, which was influenced by the distance to the edge of the water. The topsoil showed the highest moisture content (26.02%) in the upper nine soil layers (from 0.2 m to 1.1 m). The difference in soil pH was small (from 7.13 to 8.09). Both the middle (from −0.2 m to 0.3 m) and bottom layers (from −1.0 m to −0.7 m) showed high pH, and no significant difference in the horizontal direction was found. The concentrations of TN in the top (from 0.8 m to 1.1 m) and middle layers (from −0.5 to −0.1 m) were significantly higher than those in the other depths, and the mean concentrations within the initial 1.0 m from the water edge were lower than those in farther soils. The concentration of NH_4-N showed similar spatial distribution patterns of the TN, which contained 0.4%–1.9% of TN. The concentration of NH_4-N was also higher (up to 9.5 mg/kg) in the top (from 0.8 m to 1.1 m) and middle soil depths (from −0.5 m to −0.1 m) compared to other depths. The trend of NO_3-N concentration did not agree with those of TN and NH_4-N. The distribution of NO_3-N concentration showed a significant zonation pattern across the soil depth gradient above the lake surface, which gradually decreased from the maximum concentration of 19.79 mg/kg in the upper (from 1.0 m to 1.1 m) soil depth to a minimum concentration of 1.35 mg/kg at a depth of 0–0.1 m. The NO_3-N concentration decreased to 0.3–1.1 mg/kg below the lake surface, which was less than 5% of the top layers (0.8–1.1 m). The top (0.7–1.1 m) and middle layers (from −0.6 to −0.1 m) showed higher TOC concentrations, which was similar to the distribution of TN and NH_4-N. The TOC concentration in the slope was much lower than that in other sites.

Table 1 Physicochemical properties of soil samples in raised fields in Baiyang Lake

Property	Mean±SD	Range
Temperature (℃)	28.7±2.4	22.3–33.4
Moisture content (%)	27.43±5.30	18.62–42.61
pH	7.74±0.21	7.13–8.09
TN (%)	0.116±0.072	0.019–0.317
NH_4-N (mg/kg)	8.45±3.20	4.16–18.97
NO_3-N (mg/kg)	3.76±5.14	0.30–23.90
TOC (g/kg)	3.20±1.65	0.67–7.69

Notes: TN is total nitrogen; NH_4-N is ammonia nitrogen; NO_3-N is nitrate nitrogen, and TOC is total organic carbon

The rhizome biomass of *P. australis* for each distance and soil depth are shown in Fig. 4. The rhizomes of *P. australis* grew up to 1.5 m deep at the sampling sites in the raised fields, and most of them spread densely in the top 1.0 m layers. The rhizome biomass sharply decreased at the soil depth above 1.0 m.

The mean rhizome biomass in surface soils reached 1127 g/m^2, and no obvious difference was observed with the changes in the distance to the water edge. From the topsoil down to the fifth soil layer, the rhizome biomass gradually decreased to less than 20% of the initial rhizome biomass. A peak value of rhizome biomass was found in the soil depth of 0.4–0.5 m, which was 1.12 times the weight of the biomass in the topsoil. The mean rhizome biomass then sharply decreased from 1019 g/m^2 (0.3–0.4 m) to 2 g/m^2 (from −0.5 m to −0.4 m). The rhizome biomass at the slope of the raised fields

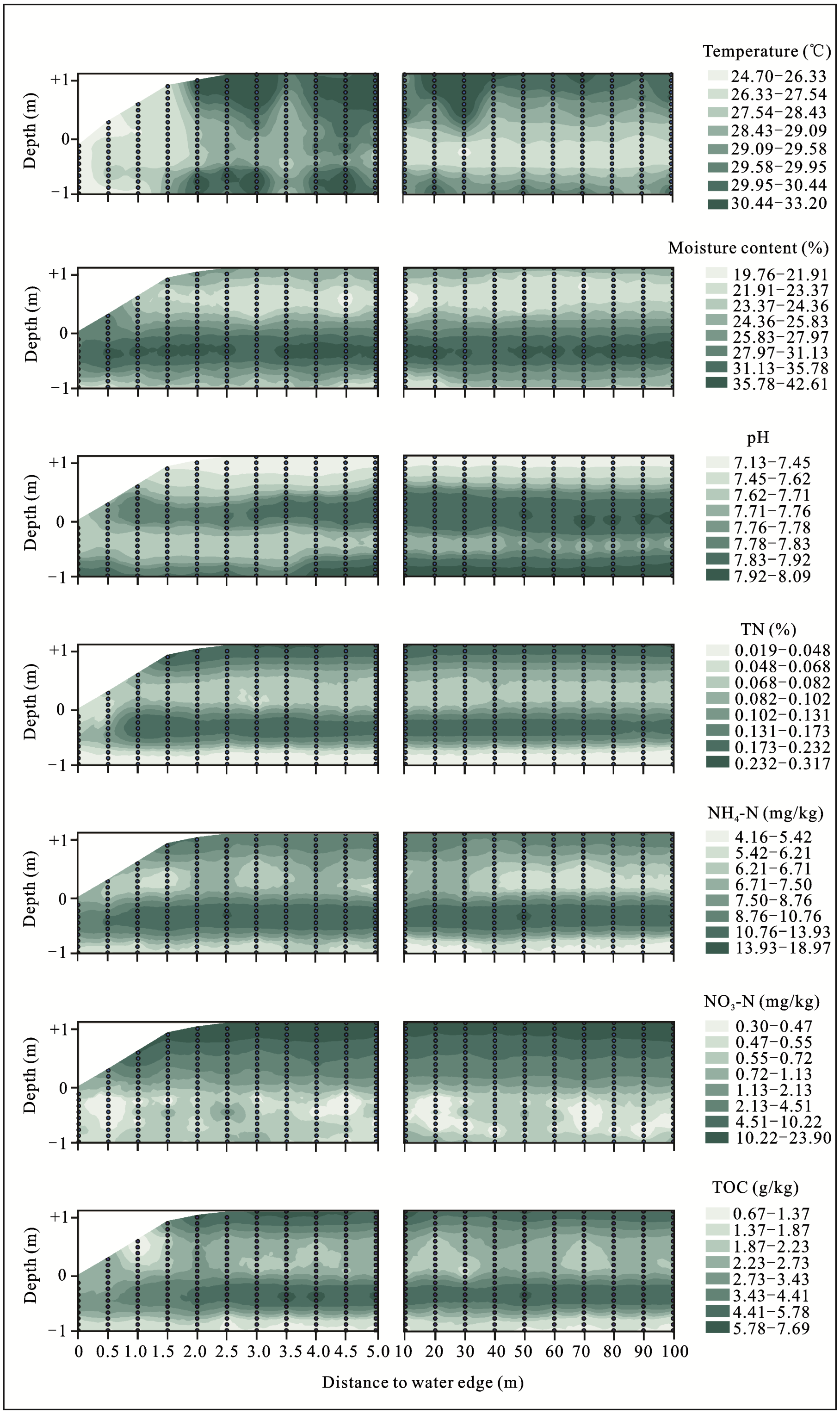

Fig. 3 Spatial distribution patterns of soil physicochemical characteristics in raised fields

was obviously higher than those farther than 4 m in the horizontal direction, probably due to the need for a higher stability of the plant growth in the slope.

A pearson correlation analysis was performed to determine the relationships between the soil position and characteristics of soil and plant (Table 2). The mean values of soil properties and rhizome biomass of *P. australis* at each depth and distance were used to better

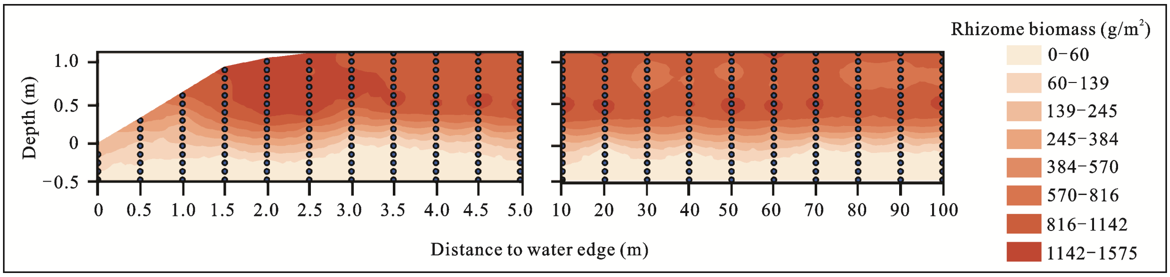

Fig. 4 Spatial distribution patterns of rhizome biomass of *P. australis* in raised fields

Table 2 Pearson correlation coefficients between soil position and characteristics of soil and plant

	Temperature	Moisture content	pH	TN	NH_4-N	NO_3-N	TOC	Rhizome biomass
Soil depth	-0.691^{**}	0.415	0.642^{**}	−0.405	−0.033	-0.826^{**}	−0.327	-0.897^{**}
Distance to water edge	−0.273	−0.062	0.593^{**}	0.197	−0.341	0.431	0.252	0.170

Note: **, correlation is significant at the 0.01 level

analyze their correlation with such factors and reduce the amount of data needed. The NO_3-N concentration ($p < 0.01$), and rhizome biomass of *P. australis* ($p < 0.01$) showed significantly negative correlation with the soil depth, while the pH showed significantly positive correlation with the soil depth ($p < 0.01$). However, the effects of soil depth on the soil temperature, moisture content, TN, NH_4-N, and TOC were insignificant ($p > 0.05$). The pH also showed a highly significant correlation with the distance to the water edge ($p < 0.01$), with a correlation coefficient of 0.593. However, no significant correlation between the distance with other environmental factors ($p > 0.05$) was found.

3.2 Spatial distribution of soil DEA across soil profiles

Figure 5 shows the spatial distribution of the soil DEA across the soil profiles in the raised fields. The DEA in all the soil samples varied between 133 ng N_2O-N/(g·h) and 2685 ng N_2O-N/(g·h) at a soil temperature of 25℃. The mean values of DEA at each depth and distance were first calculated to analyze the relationships between the DEA and soil depth, and the DEA and the distance to the water edge. The highest DEA was found in the upper soil layer (1.0–1.1 m), and did not significantly differ among the different distances. A significant decrease in the DEA was observed from the topsoil to fourth layer; below 0.7 m, it decreased slowly. The middle layers (from –0.4 m to 0 m) also showed higher values, with a wide range from 675 N_2O-N/(g·h) to 1060 ng N_2O-N/(g·h), which was higher than that in the adjacent soils except for the top layers (0.8–1.1 m). The soil DEA decreased to 133–254 ng N_2O-N /(g·h) in the deep soil layers (from −1.0 to –0.6 m).

The DEA showed significant negative correlations with the soil depth ($p < 0.01$); however, it did not show significant correlation with distance increment ($p > 0.05$) (Fig. 6). The linear regression model was used to

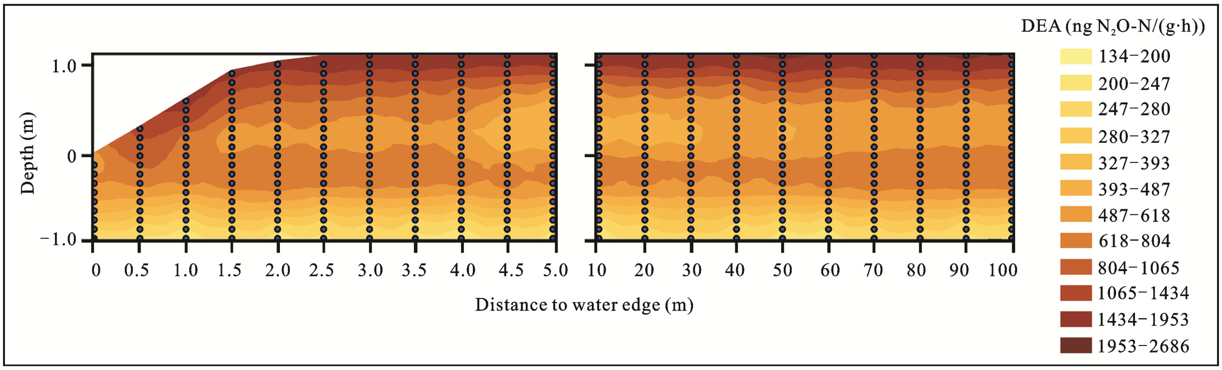

Fig. 5 Spatial distribution patterns of soil denitrification enzyme activity (DEA) in raised fields

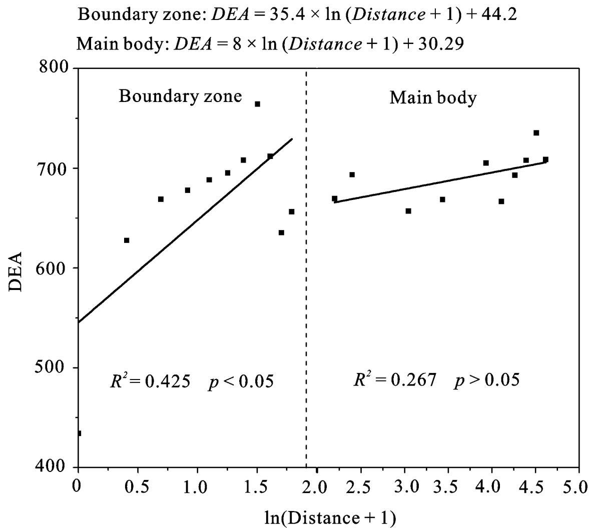

Fig. 6 Relationships between soil denitrification enzyme activity (DEA) and distance in boundary zone and main body of raised fields

further illustrate the relationships between soil DEA and distance. The DEA showed significant positive correlations with the distance to the water edge in the boundary zone of the raised fields ($p < 0.05$), while it showed no significant correlation in the main body of the raised fields ($p > 0.05$).

Pearson correlation coefficients between the soil DEA and distance were analyzed using all the data to further study the characteristics of spatial distribution of the soil DEA in the boundary zone and main body of the raised fields (Table 3). The calculation of the average values of the DEA at each distance might avoid the variation rules of the DEA at different soil depths, which significantly influenced the soil DEA. The soil DEA showed significant correlation with the distance in the upper layers of the boundary zone of the raised fields (0.2–0.7 m). No significant correlation between the DEA and distance in the surface (0.8–1.1 m) and the lower soil layers in the boundary zone of the raised fields (from –1.0 m to 0.2 m) was found. No significant relationship between the DEA and distance in the main body of the raised fields was found.

3.3 Relationships between DEA and environmental factors

The linear regression results for DEA and environmental factors are shown in Fig. 7. The soil DEA showed significant positive correlations with soil TN, NO_3-N, NH_4-N, and TOC content ($p < 0.001$), while it showed significant negative correlation with soil pH ($p < 0.001$). The highest coefficient of determination (R^2) of linear regression was found between DEA and NO_3-N ($R^2 = 0.612$). The rhizome biomass of *P. australis* also significantly correlated with DEA ($R^2 = 0.174$, $p < 0.001$). No significant correlation was observed between the DEA and soil moisture content, and DEA and soil temperature ($p > 0.05$). The soil DEA still showed significant positive correlations with soil TN, NO_3-N, NH_4-N when the results of soil samples above and below the water level were calculated by the linear regression model, respectively ($p < 0.001$).

Table 3 Pearson correlation coefficients between soil DEA and distance within each soil depth

	DEA boundary zone	DEA main body		DEA boundary zone	DEA main body
Distance (depth 1.0 to 1.1 m)	0.516	0.160	Distance (depth –0.1 to 0.0 m)	–0.559	–0.233
Distance (depth 0.9 to 1.0 m)	–0.383	0.353	Distance (depth –0.2 to –0.1 m)	0.523	–0.077
Distance (depth 0.8 to 0.9 m)	–0.556	–0.115	Distance (depth –0.3 to –0.2 m)	–0.170	0.371
Distance (depth 0.7 to 0.8 m)	–0.288	–0.003	Distance (depth –0.4 to –0.3 m)	–0.023	0.054
Distance (depth 0.6 to 0.7 m)	-0.849^{**}	0.469	Distance (depth –0.5 to –0.4 m)	0.122	0.557
Distance (depth 0.5 to 0.6 m)	-0.700^{*}	0.355	Distance (depth –0.6 to –0.5 m)	0.019	0.370
Distance (depth 0.4 to 0.5 m)	-0.792^{*}	–0.318	Distance (depth –0.6 to –0.7 m)	0.320	0.071
Distance (depth 0.3 to 0.4 m)	-0.895^{**}	0.266	Distance (depth –0.8 to –0.7 m)	–0.578	–0.428
Distance (depth 0.2 to 0.3 m)	-0.652^{*}	–0.454	Distance (depth –0.9 to –0.8 m)	0.347	0.063
Distance (depth 0.1 to 0.2 m)	–0.558	0.602	Distance (depth –1.0 to –0.9 m)	0.291	–0.520
Distance (depth 0.0 to 0.1 m)	–0.321	0.369			

Notes: *, correlation is significant at the 0.05 level; **, correlation is significant at the 0.01 level

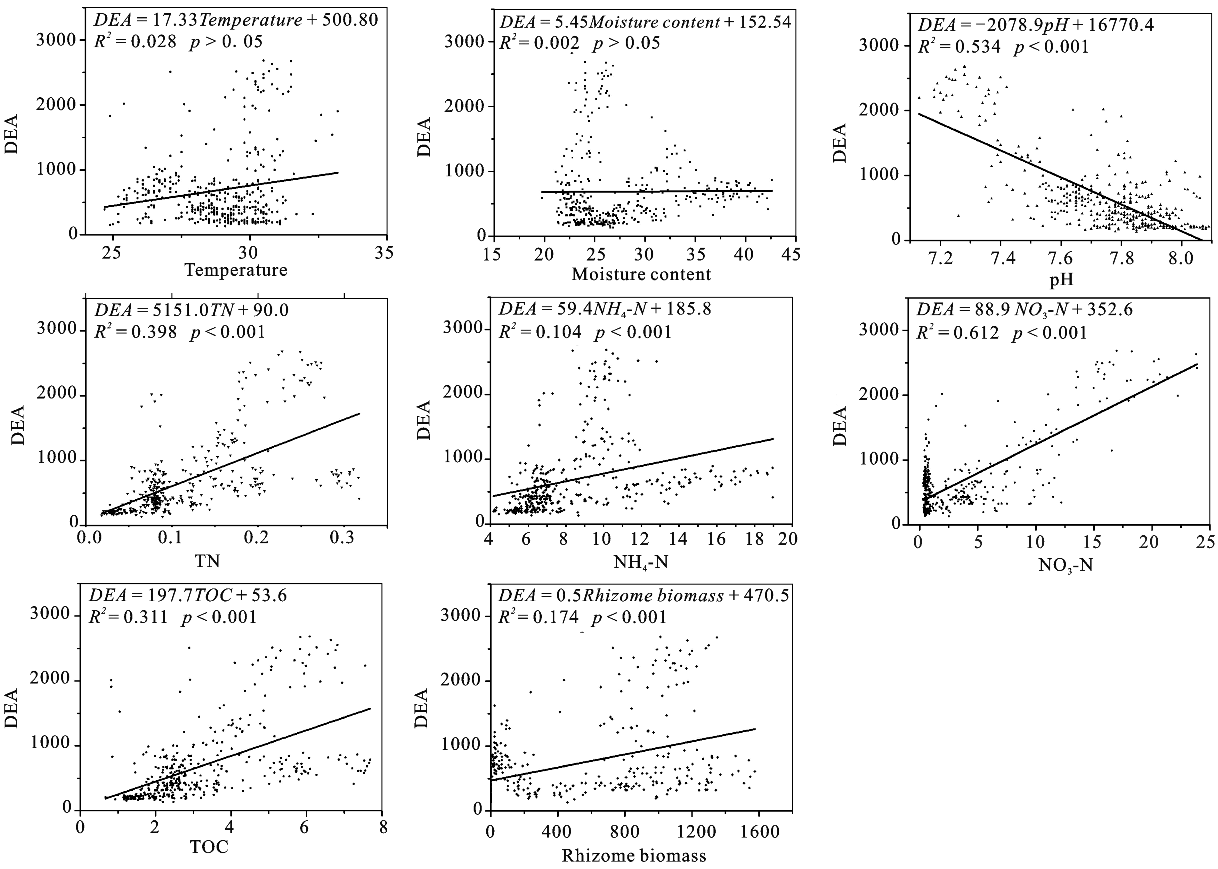

Fig. 7 Relationships between soil DEA and their environmental factors in raised fields

4 Discussion

4.1 Spatial changes in physicochemical properties of soil

The soil depth and the distance to the water edge were together used to determine the spatial distribution of physicochemical properties of soil. No significant correlations were observed between the soil nutrients and the distance to the water edge (Table 2) because of the similar vegetation compositions and plant productivity in the raised fields. The pH of the soil was the only parameter that showed significant relationship with the distance ($p < 0.01$) and is affected not only by the soil parent materials but also by tillage management, quality of plant litter, heavy metal content and base saturation of soils (Helyar *et al.*, 1990). Soil near the water edge is affected more by the lake water of low pH (pH = 7.57 ± 0.15) compared to the distant soil (Fig. 3). Further, the linear regression analysis showed significant negative correlation between soil pH and NH_4-N ($p < 0.05$). Nye (1981) reported that NO_3-N tends to raise the pH, whereas NH_4-N lowers the pH.

Concentration of the soil nutrients changed significantly with soil depth (Table 2). Bai *et al.* (2012) reported that TOC and TN contents were significantly correlated with soil depth. The results of Fig. 3 show that the soil nutrients have higher concentrations in the upper layer of top soil because of the large accumulation of plant and animal residues (Hefting *et al.*, 2004; Bai *et al.*, 2005). The concentration decreased with increasing soil depth in the area above the lake surface. The polluted lake water was considered as an important source of nutrients in land-water ecotones having a strong filtration effect, thus resulting in peak concentration of soil nutrients below the lake surface (Risser *et al.*, 1990). The excessive nutrient-rich pollutants were introduced into the Baiyang Lake by industrial waste emptied upstream, waste from population in surrounding area, water transportation, tourism, and aquaculture (Xu *et al.*, 2011), thus resulting in appearance of two peaks of TN, NH_4-N, and TOC concentrations in the raised fields in the Baiyang Lake.

Further, the result of previous study showed that soil nutrients were strongly influenced by particle-size fractions (Jolivet *et al*., 2003). Centuries ago, raised fields as a typical semi-natural agricultural system have been developed in the Baiyang Lake. Canal sediments were dredged and covered at the top of raised fields by farmers to improve the nutrients on the planting surface every year before 1990s (Wang *et al*., 2010). Therefore, the soil particle-size distribution of raised fields was significantly disturbed by human beings leading to great differences in the measurement of the particle-size fractions of soil samples; however, no obvious distribution characteristics were observed in the horizontal and vertical directions. Moreover, no significant correlation was observed between particle-size fractions and soil nutrients.

4.2 Spatial changes of rhizomes of *P. australis*

Extensive and strong rhizomes of *P. australis* were found in the raised field soil. Rhizome dynamics is determined by growth characteristics (plant varieties and rhizome age) and environmental conditions (water level, soil texture, and seasonal changes) (Karunaratne *et al*., 2004; White *et al*., 2007). Although, rhizomes covered the entire soil profiles at depths less than 1.5 m in raised fields, rhizomes growth showed the characteristics of randomness and anisotropy (Fig. 4). Rhizomes in raised fields were much deeper than those in flooded area. The lower limit of depth of rhizomes is decided by the decrease in the atmospheric supply of CO_2 and O_2 (Rea, 1996). The decomposition of root residues releases nutrients into soil, thus increasing the concentration of soil nutrients (Goulder, 1990; Wang and Yin, 2008). The spatial distribution of soil TOC was similar to the rhizome distribution in most instances; however, there was no significant correlation between rhizome biomass and TOC content in raised fields. The high TOC content would be lowered by leaching due to the downward displacement of rainwater and by the change in the water level.

Rhizome biomass showed significant positive correlation with soil depth, however, no significant correlation was observed between rhizome biomass and distance to the water edge. The surface soil contained 29.5%–40.4% of the total biomass of rhizomes in the initial 1.0 m depth of raised fields in vertical direction, and then decreased to 10.9%–15.5% in the soil profiles having increased length of the rhizomes. The percentage of rhizome biomass of *P. australis* in the surface below 10 cm of raised field soil was much lower compared to aquatic macrophytes (Caffrey and Kemp, 1991). With the appearance of a multitude of horizontal rhizomes, rhizome biomass reached a higher value in soil depth of 0.4–0.5 m than in the surface soil (Fig. 4). Moreover, a high level of rhizome biomass was observed in the slope of raised fields because of the requirement of extensive root systems to keep *P. australis* growing normally (Rhee *et al*., 2008).

4.3 Effects of environmental determinants on spatial distribution of DEA

The soil DEA varied widely from 134 ng N_2O-N/(g·h) to 2686 ng N_2O-N/(g·h), based upon the spatial location within the raised field soil in summer (Fig. 5). The results of this study showed that the relatively high DEA (> 600 ng N_2O-N/(g·h)) was found at the upper soil layers from 0.4 m to 1.1 m depth and the middle soil layers from –0.4 m to 0 m depth; however, it did not differ significantly between the different distances. Root associated carbon production and water level fluctuations improve enzyme activity. Long rhizomes of *P. australis* spread the carbon to deeper soil layer expanding the scope of denitrification process. High DEA observed in the deeper soil layers revealed the importance of subsurface denitrification in carbon-enriched soil horizons (Dhondt *et al*., 2004). Most studies were focused on the denitrification process in the top soil layers (< 100 cm) not emphasizing the NO_3-N removal by denitrification. The result of this study revealed the importance of denitrification in the deeper layers of raised fields controlling the NO_3-N content in groundwater in the Baiyang Lake.

The soil DEA immediately responded to the changes in soil conditions because extracellular soil enzyme activity was directly affected by soil physicochemical properties (Geisseler and Horwath, 2009). Soil TN, NO_3-N, and TOC contents were the most important factors affecting the spatial distribution of DEA in raised fields (Fig. 7). The highest DEA was encountered in the surface soil because of the sufficient carbon and nitrate available for denitrification enzyme expression. Stevenson *et al.* (2011) and Arce *et al.* (2013) came to the same conclusion on the relationships between denitrification and soil properties. The soil DEA had significant positive correlation with rhizome biomass which indirectly influences the denitrifying bacteria and soil properties (Ge *et al*., 2011). Priha *et al.* (1999) reported that plant roots increased the DEA in the rhizospheres, because of the extra carbon resource and anaerobic conditions caused by root respiration.

5 Conclusions

This study analyzed the spatial distributions of soil properties, rhizome biomass of *P. australis*, and soil DEA. Further, the relationships between DEA and environmental factors in the raised fields in the Baiyang Lake were established. The vertical distribution of each index obviously shows a significant zonation pattern; however, slight differences in each distance to the water edge are observed. The soil TN, NH_4-N, TOC, and DEA show two peaks in the top and middle layers, which may be caused by plant and animal residues at the upper soil layers and the polluted nutrients in lake water. The spatial distribution of the soil DEA shows the importance of subsurface denitrification in the raised fields. The linear regression analysis shows that the soil TN, NO_3-N, and TOC contents are the most important soil factors affecting the spatial distribution of DEA in the raised fields. Further research is required to investigate the temporal patterns of DEA and calculate the boundary purification effect of the raised fields under water level fluctuation conditions.

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