Elemental stoichiometry (C, N, P) of soil in the Yellow River Delta nature reserve: Understanding N and P status of soil in the coastal estuary

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HIGHLIGHTS

- Soil C, N and P contents and their stoichiometry ratios showed high spatial heterogeneity at a reserve scale.
- N limitation and a well-constrained ratio $C_{65}:P_1$ were observed in soils in the Yellow River Delta Nature Reserve.
- Longer reclamation and fertilization history decreased C:N ratio of coastal estuary soils.
- C:N and N:P ratios could be good indicators of soil development in the coastal estuary.

GRAPHICAL ABSTRACT
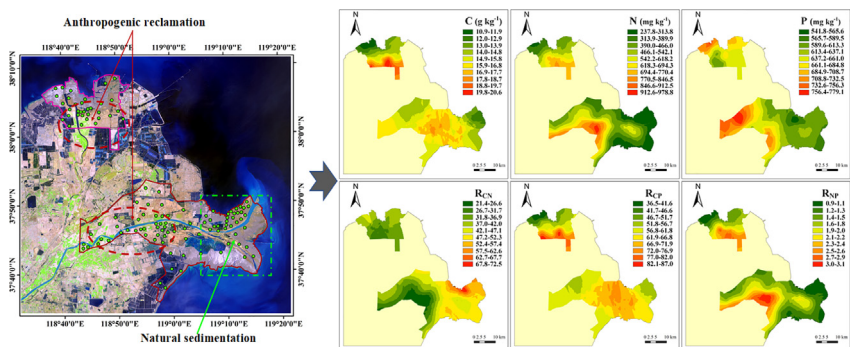
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ABSTRACT

The Yellow River Delta Nature Reserve (YNR), which includes two separated regions: part of the old Yellow River Delta (OYD) and part of the current Yellow River Delta (CYD), was established to protect coastal wetlands in the coastal estuary. A total of 120 plots were sampled in the YNR in April 2016, and the spatial patterns of soil C, N and P contents and their stoichiometric ratios (C:N (R_{CN}), C:P (R_{CP}) and N:P (R_{NP})) were studied and interpolated using the Ordinary Kriging method. Results indicated that the soil elemental contents and stoichiometric ratios showed high spatial heterogeneity and large variations. The mean C:N:P ratio (R_{CNP}) was ~ 64.7:2.3:1 in OYD, and ~ 64.5:2.0:1 in CYD, respectively, and a well-constrained R_{CP} ratio ~ 65:1 was found in the 0–50 cm soil depth within the YNR. N showed greater variation than C and P. Furthermore, N contents in the 0–5 cm soil layer of OYD were significantly higher than that of CYD (F = 4.79, p = 0.03); R_{CN} in 0–5 cm, 5–10 cm layers of OYD was significantly lower than those in the same layers of CYD (F = 4.75, p = 0.03; F = 5.18, p = 0.02, respectively). R_{NP} in 0–5 cm soil layer of OYD was notably higher than that of CYD (F = 4.88, p = 0.03). These results were due to the combined actions of sedimentation, reclamation and fertilization. Finally, we concluded that a longer reclamation and fertilization history led to decreased R_{CN} in coastal estuary soils, confirmed that the soil of the YNR exhibits N limitation, and suggested that the soil R_{CN} and R_{NP} could be good indicators of the anthropogenic improvement status during soil development in this coastal estuary.

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1. Introduction

All organisms and ecosystems are composed of a mixture of multiple chemical elements with certain proportions that vary with the study context (Falkowski and Davis, 2004; Loladze et al., 2000; Michaels, 2003; Sterner and Elser, 2002). Carbon (C), nitrogen (N) and phosphorus (P) are the most common elements and key macronutrients that play a major role in the metabolism and nutrient cycles of natural ecosystems (Michaels, 2003; Pujo-Pay et al., 2011). Ecological stoichiometry (ES) is to study the balance of multiple chemical substances in ecological interactions and processes in ecosystems, particularly on C, N and P (Michaels, 2003; Sterner and Elser, 2002; Hessen et al., 2013; Qu et al., 2014). C, as the building block of life, makes up carbon-based lifeforms, which are the only lifeforms that exist on Earth. However, N and P limitation appears ubiquitous in terrestrial, freshwater, and marine ecosystems (Blomqvist et al., 2004; Elser et al., 2007; Manzoni et al., 2010; Qu et al., 2014; Vitousek and Howarth, 1991). Since Alfred C. Redfield (1890–1983) found that the atoms of C, N and P in plankton from the open sea are present, on average, in the ratio: $C_{106}:N_{16}:P_1$ (Redfield, 1934, 1958; Gruber and Deutsch, 2014), ES, as a fundamental principle that supports our understanding of marine biogeochemistry, has served as a powerful organizing principle of biogeochemical research for illustrating how biological processes occur at different ecosystem levels (Falkowski and Davis, 2004; Michaels, 2003; Pujo-Pay et al., 2011). This has resulted in a search for an analogue for Redfield ratios in terms of organismal C:N:P ratios on different scales, from genes to ecosystems and from molecules to the biosphere (Cleveland and Liptzin, 2007; Elser et al., 2000a; Michaels, 2003; Sardans et al., 2012; Sterner and Elser, 2002).

To date, ES has put the principles of conservation of mass and energy, which all organisms follow, to new ecological uses (Hessen et al., 2013; Sterner and Elser, 2002). At the organismal level, the biochemical composition reflects evolutionary outcomes that influence the growth rate, fitness, metabolism, structure, and other aspects of ecological success (Michaels, 2003; Sterner and Elser, 2002). ES can also be effectively used to explain biological productivity, nutrient constraints and restrictions, homeostatic regulation and evolution of ecosystem, and fluxes of mass and energy in chemical reactions at the ecosystem and biosphere levels (Elser et al., 1998; Loladze et al., 2000; Yu et al., 2010). Up to date, abundant studies indicate that ES is a powerful tool that is valuable in understanding the relationships of organisms and ecosystem structure and function to the environment and to organismal stoichiometry (Müller et al., 2017; Michaels, 2003; Pujo-Pay et al., 2011; Qu et al., 2014; Vitousek and Howarth, 1991; Bradshaw et al., 2012). Although there have been great advances in the field of ES, related researches mainly focused on C, N and P ecological stoichiometry of the dominant species or invasive species in the coastal estuaries, and on some of the specific factors, such as, agricultural land use and flooding intensity, which influence on C, N and P stoichiometry of soil or plant in the coastal wetlands in China, (Feng et al., 2019; Sun et al., 2014; Zhang et al., 2013; Wang et al., 2015a, 2015b). Field study of elemental stoichiometry in the soils, which take a nature reserve as a whole study object, is unfortunately very scarce.

Coastal estuaries usually include wetlands with one or more rivers or streams flowing into the ocean normally and are semi-enclosed by land that is also exposed to a mixture of fresh and brackish water bodies (Cowardin et al., 1979). Coastal wetlands are responsible for approximately half of all C burial in oceans, and their persistence as a valuable ecosystem depends largely on their ability to accumulate organic material at a rate equivalent to the relative sea level rise (Kirwan and Blum, 2011). The combustion of fossil fuels, the production of fertilizers, agriculture, and other anthropogenic activities have greatly increased the transfer of N and P through rivers to estuaries and coastal oceans (Bennett et al., 2001; Vitousek et al., 1997). Whether N and P accumulation or limitation occurs in coastal wetlands substantially changes the composition and functioning of estuarine and nearshore ecosystems (Peñuelas et al., 2012; Sundareshwar et al., 2003). There is a clear need for us to study the nutrients status of C, N and P and their elemental stoichiometric characteristics in soil, to better understand the N or P limitation in the coastal estuary. In this paper, we hypothesized that a similar Redfield ratio and the paradigm of N limitation in the coastal wetlands exists in soils in the Yellow River estuary. Therefore, we choose the nature reserve in the Yellow River Delta (YRD) as a study area. Our study objectives are to: 1) inventory the range of variation in soil C, N and P contents and stoichiometric ratios; 2) produce spatial distribution maps of the contents of C, N and P and the stoichiometric ratios of C:N (R_{CN}), C:P (R_{CP}) and N:P (R_{NP}); 3) verify whether a well-constrained C:N:P ratio (R_{CNP}) exists in the soil in the YNR; and 4) understand N or P limitation in soil in this coastal estuary.

2. Material and methods

2.1. The YRD and its nature reserve

The YRD, which was formed from a large crevasse that begins at Lankao, Henan Province and that made the Yellow River re-enter the Bohai Sea in northern Shandong Province in 1855 (Xue, 1993), is located at the south shore of the Bohai Sea and the western part of Laizhou Bay (Fig. 1). The course of the Yellow River has shifted many times, and six main courses have existed in succession after 1934: the Songchunronggou course (1934–1953), the Tianshuigou course (1934–1953), the Shenxiangou course (1953–1964), the Diaokou course (1964–1976), the Qingshuigou course (1976–1996), and the Q8 course (1996-) (Zhang et al., 2018). The delta is not only the most active region of land-ocean interaction and one of the largest river deltas in the world (Yang et al., 2016), but also the youngest coastal wetland ecosystem in the warm-temperate zone in China, with immature, fragile and unstable characteristics (Li et al., 2009; Bai et al., 2012; Xie et al., 2016). The accretion rate by the land extension of the delta before 1979 was 32.4 km² yr⁻¹, and 2.7 km² yr⁻¹ after 1979 because of the channel diversion of the Yellow River and the decrease of flow and sediment in the estuary (Li et al., 2009). From 1976 to 2015, the sediment load of the Yellow River was reduced from 898 M tons to 31 M tons per year; these data were recorded by the Lijin hydrological station (Zhang et al., 2018).

The study area is the Yellow River Delta Nature Reserve (YNR) (Fig. 1), which was established to protect newly formed coastal wetlands, rare and endangered birds, and their important habitats, breeding and stopover sites in 1992 by the State Council of China. The YNR consists of two parts: the north part, which is part of the old Yellow River Delta (OYD) and was formed when the Yellow River followed the Shenxiangou and the Diaokou courses flowing into the Bohai Sea between 1953 and 1976; and the south part, which is part of the current Yellow River Delta (CYD) and was mainly formed after an artificial diversion of the river course to the Qingshuigou course in 1976, to prevent the degradation and erosion that occurred in the Songchunronggou and the Tianshuigou courses. In the meantime, a rapid and discontinuous decrease in water and sediment discharge resulted in coastal estuarine wetland degradation, seawater infiltration, and the formation of a secondary saline-alkali area in OYD, because of the artificial course change (Li et al., 2009).

The study area belongs to the warm temperate continental monsoon climate zone, with four distinct seasons, hot and rainy summers, cold and dry winters, an average annual temperature of 12.1 °C and an average annual precipitation of 555.9 mm between 1981 and 2010. The soil in the study area is classified as saline alluvial soil which was formed by the deposition of loess (Fluvisols, FAO). *Phragmites australis* (Cav.) Trin. ex Steud., *Tamarix chinensis* Lour., *Suaeda heteroptera* Kitag. and *Imperata cylindrica* (L.) Beauv. are the common natural plant species in the coastal marsh, meadow, and tidal wetlands, and locust forests (*Robinia pseudoacacia* Linn.), cotton fields (*Gossypium hirsutum* Linn.), wheat (*Triticum aestivum* L.) and paddy fields are the predominant artificial cultivation types in the YNR. Meanwhile, a thematic land use map
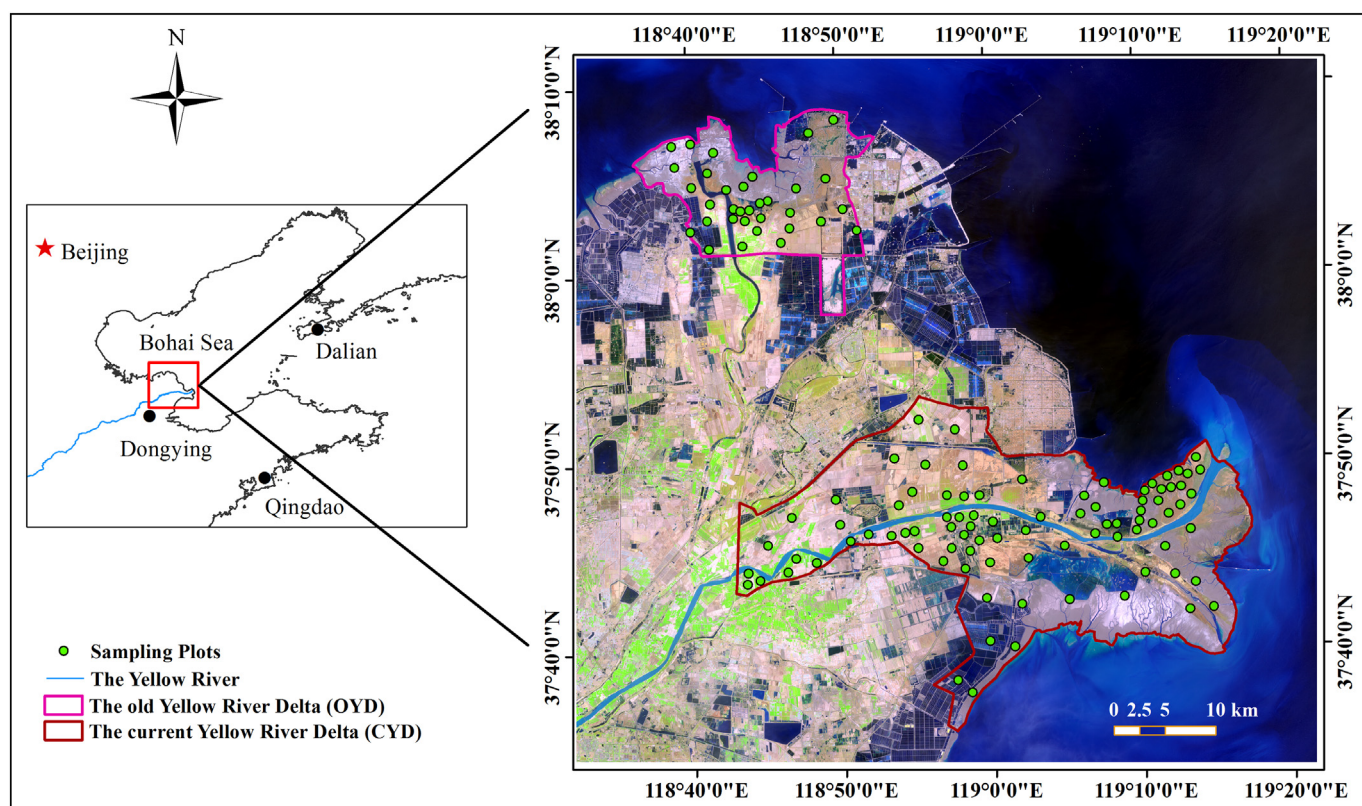
Fig. 1. Location of the sampling plots in the YNR, China.

of the YNR (Fig. S1), which is classified by means of geographical knowledge and visual interpretation, was generated to demonstrate the detail information.

2.2. Soil collection and statement

To understand the N or P limitation in soil in the YNR in the Yellow River estuary, with due consideration of the status of land conservation and utilization, the types of vegetation cover and the accessibility of sampling sites, a total of 120 plots were sampled in April 21–30, 2016, with 33 OYD plots and 87 CYD plots. In each plot, 5 replicate soil columns were collected using a stainless-steel slide hammer within a circle with a 2 m radius. Each collected column was sectioned at 5, 10, 20 cm with a 50 cm final depth and then mixed the soil samples with the same layer to get 0–5, 5–10, 10–20, 20–50 cm layers' soil samples. The soil samples were stored in zip-lock polyethylene plastic bags immediately after collection. The air-dried soil samples were ground using a mortar and pestle, and then sieved through 0.850-mm and 0.149-mm sieves for laboratory analysis.

We state that we obtained scientific research permits for these locations and activities. At the same time, we confirm that the soil collection did not involve or influence endangered or protected species.

2.3. Chemical assay and data analyses

The methods used to determine the total C, total N and total P of soil have been previously well described (Qu et al., 2014). The elemental stoichiometry (C, N, P) were calculated as atomic ratios after the conversion from mg kg^{-1} to mmol kg^{-1} from the soil total C, N and P concentrations. Difference tests of the significance of the C, N and P concentrations and stoichiometric ratios within groups were analyzed by the SPSS 16.0 software packages (SPSS Inc., 2008). The data about the contents of C, N and P, and the stoichiometric ratios of R_{CN}, R_{CP}

and R_{NP} were processed and interpolated using the Ordinary Kriging method, and the spatial distribution maps were generated using a topographic map for the studied area. Kriging spatial interpolation and mapping techniques were performed using ArcGIS 10.3 (Esri Inc., 2018). Other figures were drawn by using the OriginPro 9.1 software packages (OriginLab Inc., 2018).

3. Results

3.1. Soil C, N and P contents and their stoichiometry

The soil C, N and P contents and stoichiometric ratios in the soil profiles of the YNR are listed in Table 1. The content of C ranged from 8.8 to 37.5 g kg^{-1} with a coefficient of variation (c.v.) of 0.32; that of N from 168.0 to 3321.8 mg kg^{-1} with a c.v. of 0.74; and that of P from 534.3 to 1045.3 mg kg^{-1} with a c.v. of 0.13 in soils in OYD. The content of C ranged from 7.8 to 33.7 g kg^{-1} with c.v. of 0.30, that of N from 82.2 to

Table 1
Classical analyses of the soil C, N and P contents and their stoichiometric ratios in the YNR.

Areas	Parameters	Max	Min	Mean	Std	c.v.
OYD ($n = 132$)	C (g kg^{-1})	37.5	8.8	16.9	5.4	0.32
	N (mg kg^{-1})	3321.8	168.0	717.3	532.5	0.74
	P (mg kg^{-1})	1045.3	534.3	680.2	86.3	0.13
	R_{CN}	95.4	12.4	35.7	15.9	0.44
	R_{CP}	133.3	26.5	64.7	20.7	0.32
	R_{NP}	10.0	0.5	2.3	1.6	0.69
CYD ($n = 348$)	C (g kg^{-1})	33.7	7.8	16.6	5.0	0.30
	N (mg kg^{-1})	2110.2	82.2	635.0	435.8	0.69
	P (mg kg^{-1})	1121.8	449.3	665.8	114.1	0.17
	R_{CN}	158.8	15.3	43.9	27.0	0.62
	R_{CP}	123.8	32.8	64.5	18.0	0.28
	R_{NP}	7.0	0.2	2.0	1.3	0.62

2110.2 mg kg^{-1} with a c.v. of 0.69, and that of P from 449.3 to 1121.8 mg kg^{-1} with a c.v. of 0.17 in soils in CYD. The c.v.s of soil C and N contents in OYD were larger than those in CYD, and c.v. of soil P content was to be the opposite. The mean C, N and P contents were 16.9 g kg^{-1}, 717.3 mg kg^{-1}, and 680.2 mg kg^{-1}, respectively, in soils in OYD and 16.6 g kg^{-1}, 635.0 mg kg^{-1} and 665.8 mg kg^{-1}, respectively, in CYD soils. Though the mean content of C, N and P in soils in OYD were higher than those of CYD, there were no significant differences in the contents of soil C, N and P between them (F = 0.35, p = 0.554; F = 3.00, p = 0.084, and F = 1.72, p = 0.189).

The range of R_{CN} was 12.4 to 95.4, R_{CP} 26.5 to 133.3, and R_{NP} 0.5 to 10.0, with c.v.s of R_{CN} 0.44, R_{CP} 0.32, and R_{NP} 0.69 in soils in OYD. While the range of R_{CN} was 15.3 to 158.8, R_{CP} 32.8 to 123.8, and R_{NP} 0.2 to 7.0, with c.v.s of R_{CN} 0.62, R_{CP} 0.28, and R_{NP} 0.62 in soils in CYD. The c.v. of R_{CN} in OYD was smaller than those in CYD, and c.v.s of R_{CP} and R_{NP} showed to be the opposite trend. The mean R_{CN} was 35.7, R_{CP} 64.7 and R_{NP} 2.3 in soils in OYD and the mean R_{CN} was 43.9, R_{CP} 64.5 and R_{NP} 2.0 in soils in CYD. The mean R_{CN} in soils in OYD was lower than those of CYD and R_{NP} showed to be the opposite trend. There was no significant difference in R_{CP} between the two areas.

3.2. Spatial distribution of C, N and P contents

In the soil profiles of the OYD, the contents of C, N and P showed surface accumulations and decreased with increasing soil depth (Fig. 2 and Figs. S2–S4). The highest mean content of C, 20.4 g kg^{-1}, appeared in the 0–5 cm soil layer and the lowest mean content of C, 14.2 g kg^{-1}, appeared in the 20–50 cm soil layer; while the mean content of N decreased from 1175.1 mg kg^{-1} in the 0–5 cm soil layer to 446.0 mg kg^{-1} in the 20–50 cm soil layer, and P from 720.4 mg kg^{-1} to 627.4 mg kg^{-1} at the same soil layers (Table 2). The N content in soils at same soil layers in OYD showed larger spatial variation than the C and P contents (Figs. S2–S4). The c.v.s of the contents of C and N in the 0–5 cm soil layer in OYD were 0.35 and 0.67, respectively, which were higher than those in the other soil layers in the soil profile, and the lowest c.v. of 0.25 and 0.43 were in the 10–20 cm soil layer. While the highest c.v. of the P content was 0.14 in the 5–10 cm soil layer, and the lowest was 0.09 in the 20–50 cm soil layer. Duncan's multiple-range testing showed significant differences in the contents of C, N and P among the different layers in OYD (p = 0.05). Difference grouped results are shown in Table 2.

The vertical distributions of C, N and P in the soil profiles of the CYD showed the same surface accumulation and downward trends as those in the OYD (Fig. 2 and Figs. S2–S4). Duncan's multiple-range test also showed significant differences in the contents of C, N and P among the different layers in CYD (p = 0.05). The highest c.v. of the C content was 0.31 in the 0–5 cm soil layer, that of N was 0.61 in the 5–10 cm soil layer, and that of P was 0.19 in the 5–10 cm soil layer; while the lowest c.v. of the C content was 0.24 in the 10–20 cm soil layer, that of N was 0.57 in the 10–20 cm soil layer, and that of P was 0.10 in the 20–50 cm soil layer in the soil profiles in CYD (Table 2). The c.v. of the soil N content showed no significant difference among the different soil layers in CYD, while the c.v. of the soil P content was higher at top layer and lower at bottom layers. The soil N content in the same soil layers in CYD also showed greater spatial variation than those of C and P in the soil profiles in CYD (Figs. S2–S4). Meanwhile, there were no notable differences in the C, N and P contents in the same layers between OYD and CYD, and significant difference existed only in the N content in the 0–5 cm soil layer between OYD and CYD (F = 4.79, p = 0.03) and the N content in the 0–5 cm soil layer in OYD was higher than that of the CYD (Fig. S3).

3.3. Spatial distribution of C, N and P stoichiometric ratios

In the soil profiles of the OYD, R_{CN} was ranked as the 0–5 cm soil layer (26.4) < the 5–10 cm soil layer (33.2) < the 10–20 cm soil layer (39.6) < the 20–50 cm soil layer (43.5); and increased with increasing soil depth (Figs. 3 and S5). Duncan's multiple-range test indicated a significant difference in R_{CN} between the 0–5 cm soil layer and the 10–50 cm soil layers, between the 0–10 cm soil layers and the 20–50 cm soil layer, while the differences between the 5–10 cm soil layer and the 10–20 cm soil layer were not significant (Table 2). R_{CP} was ranked as the 0–5 cm soil layer (73.7) > the 5–10 cm soil layer (64.7) > the 10–20 cm soil layer (61.1) > the 20–50 cm soil layer (59.3); and decreased with increasing soil depth (Figs. 3 and S6). A significant difference in R_{CP} between the 0–5 cm soil layer and the 10–50 cm soil layers was found by Duncan's multiple-range test, while the differences between the 0–5 cm soil layer and the 5–10 cm soil layer were not significant (Table 2). R_{NP} showed the same ranking as R_{CP}, and there was a significant difference in R_{NP} between the 0–5 cm soil layer and the rest soil layers (Table 2 and Fig. S7).

In the soil profiles of the CYD, R_{CN}, R_{CP} and R_{NP} showed the same order ranking as those of in OYD (Fig. 3 and Figs. S5–S7). There were significant differences in R_{CN} between the 0–20 cm soil layers and the 20–50 cm soil layer, and in R_{CP} and R_{NP} between the 0–5 cm soil layer and the 5–50 cm soil layers. While the difference in R_{CN} between the 5–10 cm soil layer and the 0–20 cm soil layers, the differences in R_{NP} between the 10–20 cm soil layer and the 5–50 cm soil layers were not significant in the soil profiles of the CYD (Table 2). R_{NP} showed larger spatial variation than R_{CN} and R_{CP} in the soil profiles in OYD, and R_{CN} and R_{NP} showed larger spatial variation than those of R_{CP} in the soil
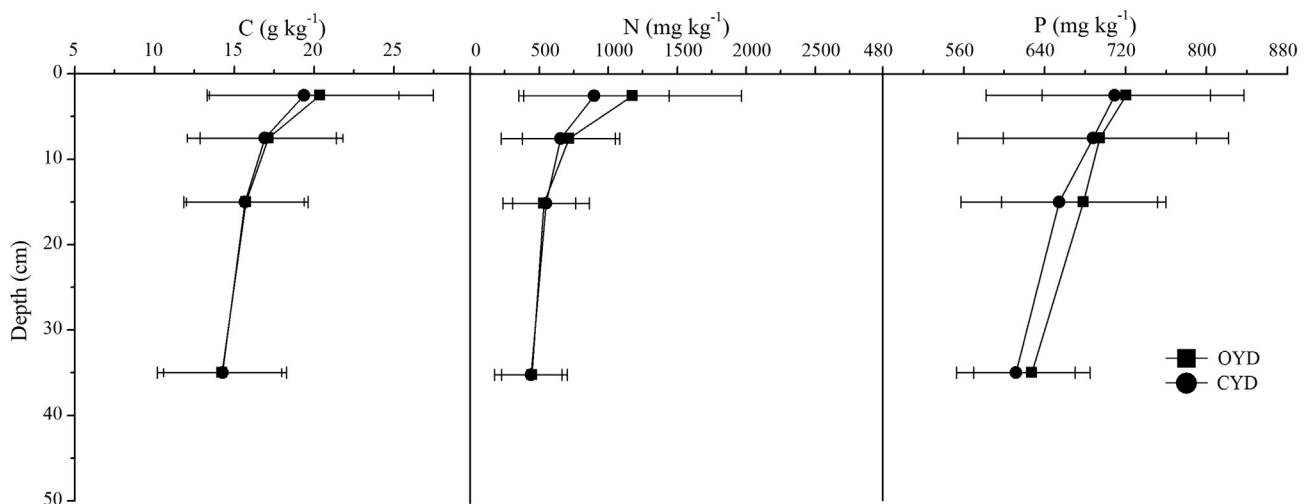

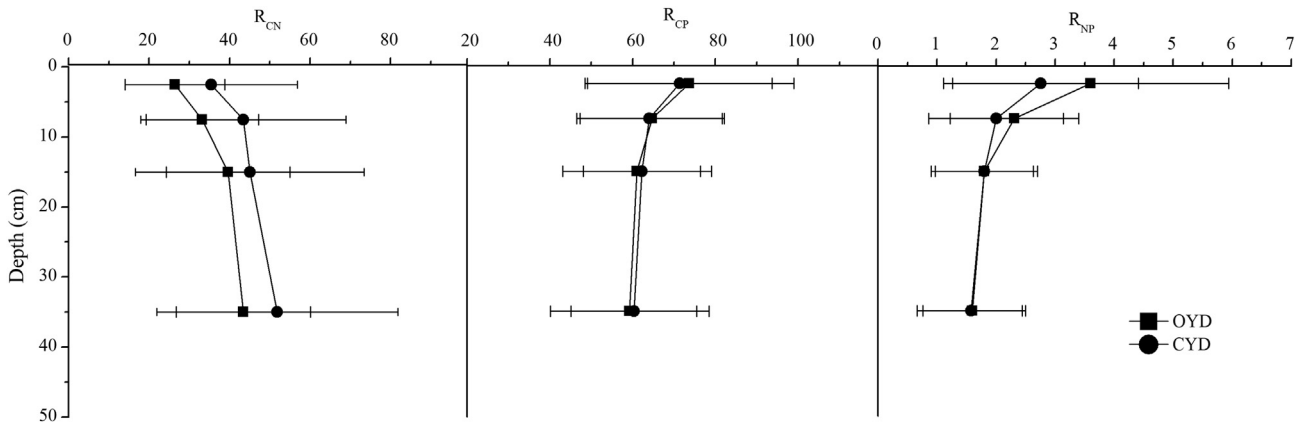**Fig. 2.** Vertical distributions of the content of C, N and P in the soil profiles of the YNR.

Fig. 3. Vertical distributions of R_{CN}, R_{CP} and R_{NP} in the soil profiles of the YNR.

profiles in CYD; and R_{CP} showed relatively narrow spatial variations in both OYD and CYD (Fig. 3 and Figs. S5–S7). There were significant differences in R_{CN} at the 0–5 cm soil layer (F = 5.18, p = .02) and at the 5–10 cm soil layer (F = 4.75, p = .03) between OYD and CYD, and the value of R_{CN} at the 0–10 cm layers of OYD was lower than that of the CYD (Figs. 3 and S5). Meanwhile, the value of R_{NP} at the 0–5 cm layer of OYD was higher than that of the CYD (Figs. 3 and S7), and there was a notable difference in R_{NP} at 0–5 cm soil layer between the two areas (F = 4.88, p = 0.03).

4. Discussion

4.1. How can we explain the differences in soil elemental stoichiometry (C, N, P) between OYD and CYD?

It is well known that the soil in the YRD is formed by the sediment load of the Yellow River, which comes from water and soil erosion on the Loess Plateau, and the Loess Plateau has had a relatively low rate of vegetation cover and a fragile environment in the past (Zhang et al., 2016a). This study presents two parts of the YNR, and describes the elemental stoichiometry (C, N, P) of the soils encountered with the diversion of the channels. Typical soil formation processes include the accumulation stage of the parent material and the differentiation stage of horizons. The parent material of a soil determines the original supply of nutrients released by weathering and influences the balance between nutrient loss and retention (Anderson, 1988). Soil formation in the YNR is evidently influenced by the complicated functions of sedimentation, plant growth, climate change, reclamation history, and other natural and artificial factors (Mao et al., 2016; Zhang et al., 2016b). The sedimentary and reclamation histories of coastal zones gradually lengthen from the sea to the inland areas (Martini and Wanless, 2014). Plants

absorb CO_2 from the atmosphere to sequester solar energy via photosynthesis and contribute large pulses of C, N, P and other nutrients into soil ecosystems through litterfall (Schlesinger and Bernhardt, 2013). Soil microorganisms such as mycorrhizal fungi and symbiotic N-fixing bacteria provide nutrients to plants in exchange for photosynthetic C (Menge et al., 2017). Soil micro- and meso-biota biomass, community structure and function are constrained by the elemental C:N:P ratios of available litter resources (Maaroufi and Long, 2020; Maaroufi et al., 2018; Martinson et al., 2008; Ott et al., 2014). In return, soil animals, which have a wide range of stoichiometric requirements, alter the stoichiometry of leaf litter entering the soil by consumption and digestion processes (Buchkowski et al., 2019). Climate extremes and drying and rewetting cycle frequencies are predicted to increase as climate change progresses (Maaroufi and Long, 2020; Reichstein et al., 2013). Climate extremes such as droughts or storms can lead to a decrease in regional ecosystem carbon stocks and therefore have the potential to negate an expected increase in terrestrial carbon uptake (Maaroufi and Long, 2020). The process of drying and rapidly rewetting soil increases the mineralization rates of C and N, the release of P due to changes in soil structure, the nutrient desorption from soil particles and the lysis of fungal and bacterial cells (Maaroufi and Long, 2020; Turner and Haygarth, 2001). However, we believe that the reclamation history played an important role in creating the differences in soil elemental stoichiometry (C, N, P) of between OYD and CYD.

The two parts of the YNR have been and are undergoing reclamation, though they were knitted together to protect newly-formed coastal estuarine wetlands, and only a small portion of the old and the current river mouth are part of real wetlands (Qu et al., 2014; Ye et al., 2006; Zhang et al., 2018). Accordingly, the plants growing on younger temperate and high-latitude soils tend to be N limited, while vegetation on older, highly weathered soils is often P limited (Manzoni et al., 2010;

Table 2
Distributions of the soil C, N and P contents and their stoichiometric ratios under different soil layers in the YNR.

Areas	Parameters	0-5 cm	5-10 cm	10-20 cm	20-50 cm
OYD (n = 33)	C (g kg^{-1})	20.4 ± 7.1 [a]	17.1 ± 4.3 [b]	15.7 ± 3.9 [bc]	14.2 ± 4.1 [c]
	N (mg kg^{-1})	1175.1 ± 789.2 [a]	714.3 ± 336.4 [b]	533.8 ± 228.7 [bc]	446.0 ± 218.8 [c]
	P (mg kg^{-1})	720.4 ± 83.2 [a]	694.4 ± 95.3 [ab]	678.4 ± 81.3 [b]	627.4 ± 57.5 [c]
	R_{CN}	26.4 ± 12.4 [c]	33.2 ± 14.0 [bc]	39.6 ± 15.3 [ab]	43.5 ± 16.7 [a]
	R_{CP}	73.7 ± 25.2 [a]	64.7 ± 17.5 [ab]	61.1 ± 18.0 [b]	59.3 ± 19.1 [b]
	R_{NP}	3.6 ± 2.3 [a]	2.3 ± 1.1 [b]	1.8 ± 0.8 [b]	1.6 ± 0.8 [b]
CYD (n = 87)	C (g kg^{-1})	19.4 ± 5.9 [a]	16.9 ± 4.9 [b]	15.7 ± 3.7 [b]	14.3 ± 3.7 [c]
	N (mg kg^{-1})	897.5 ± 544.6 [a]	654.2 ± 430.2 [b]	548.5 ± 313.7 [bc]	439.8 ± 264.3 [c]
	P (mg kg^{-1})	709.4 ± 127.6 [a]	687.9 ± 133.7 [a]	654.2 ± 97.3 [b]	611.5 ± 58.6 [c]
	R_{CN}	35.4 ± 21.4 [c]	43.4 ± 25.5 [bc]	45.01 ± 28.4 [b]	51.8 ± 29.9 [a]
	R_{CP}	71.4 ± 22.3 [a]	64.1 ± 17.6 [b]	62.2 ± 14.2 [b]	60.3 ± 15.2 [b]
	R_{NP}	2.8 ± 1.6 [a]	2.0 ± 1.1 [b]	1.8 ± 0.9 [bc]	1.6 ± 0.9 [c]

The superscript a, b and c refer to significant differences between groups, and groups in homogeneous subsets are marked as the superscript ab and bc. The order of groups with a rank as: a > ab > b > bc > c (p = 0.05, n =33 or 87).

Qu et al., 2014; Reich and Oleksyn, 2004; Zechmeister-Boltenstern et al., 2015). We found that the content of N in the 0–5 cm soil layer in OYD was significantly higher than that of CYD (Fig. 2 and Table 2), and the value of R_{CN} in the 0–10 cm layers of OYD was notably lower than that of CYD (Fig. 3 and Table 2). This finding confirms that the long reclamation history with similar cultivation and fertilization management resulted in increased nutrient accumulation and decreased C:N ratios at the surface layer in the YNR. The findings were similar to those of Velthuis et al. (2017), who found that nutrient addition consistently leads to decreased C:N ratios in submerged aquatic plants, and Maaroufi et al. (2018) reported a decline in the total microbial and fungal biomass in forest plots where half of the total N added over 16 years of simulated N deposition was retained in the soil humus, thereby reducing the humus C:N ratio. As the soil-vegetation system is a synthesis, to complete this theory, we concluded that a longer reclamation and fertilization history leads to decreased C:N ratios in coastal estuary soils.

4.2. Is there an analogous Redfield ratio at the coastal estuary scale for the YNR?

The theory of ES, which was originally derived from the "Redfield ratio" in oceanic ecosystems, has expanded greatly and gained increasing attention for a wide range of ecosystem types in recent decades (Austin and Vitousek, 2012; Hessen et al., 2013; Waal et al., 2018). ES can be applied to connect ecology with biogeochemistry and ecosystem metabolism (Welti et al., 2017). Therefore, it would also make sense to extend stoichiometric thinking to soil which is particularly useful for establishing links between different ecosystem compartments (Zechmeister-Boltenstern et al., 2015). Cleveland and Liptzin (2007) found consistent analogous patterns in both soil (C_{186}:N_{13}:P_1) and soil microbial biomass (C_{60}:N_7:P_1) in grassland and forests at the global scale. Tian et al. (2010) found a not well-constrained ratio with a mean of C_{60}:N_5:P_1 for the entire soil depth and a well-constrained ratio, C_{134}:N_9:P_1, for the 0–10 cm organic-rich soil among different climatic zones, soil orders, soil depths and weathering stages in China. Based on our previous work, Qu et al. (2014), we found that the C:P ratios showed of relatively high variation, and the average atomic N:P ratios in both the soil ($N_{1.9}$:P_1) and the plants (N_{23}:P_1) were well-constrained in three reed-dominated coastal wetlands in the YRD. However, based on an inventory data set of 120 soil profiles, our analysis showed that the mean R_{CN}, R_{CP} and R_{NP} in OYD were 35.7, 64.7 and 2.3, respectively, and 43.9, 64.5 and 2.0 in CYD. Table 2 and Fig. 4 show the descriptive statistics of the stoichiometric ratios in the YNR (maps of the entire soil depth only are presented in Fig. 4). We did find significant variation in the soil elemental contents and their stoichiometric ratios between the two parts, but the R_{CP} showed a well-constrained ratio (C_{65}:P_1) for the 0–50 cm soil profiles of the YNR, and its consistency was more apparent than the slight differences in R_{CP} (Fig. 4). The R_{CNP} in OYD and CYD were 64.7:2.3:1 and 64.5:2.0:1, respectively (Table 3).

Redfield expounded on the close correspondence between the R_{CNP} in phytoplankton biomass and in average seawater because phytoplankton tend to consume these nutrients at a relatively fixed ratio (N_{16}:P_1) during their growth (Gruber and Deutsch, 2014; Redfield, 1958). Today, the ratio of C to N and P in marine organic matter is understood to be relatively constant. However, this ratio probably varied during Earth's history as a consequence of changes in the phytoplankton community and in ocean oxygen levels (Klausmeier et al., 2004). Klausmeier et al. (2004) found that the canonical Redfield N:P ratio of 16 is not a universal biochemical optimum, but instead represents an average of species-specific N:P ratios. Although, the median structural N:P in 29 freshwater and marine areas of 17.7 is close to the Redfield ratio, the structural N:P ratios range from 7.1 to 43.3, with an outlier at 133.3. Cleveland and Liptzin (2007) found significant variation in soil and microbial element ratios between forests and grasslands in terrestrial ecosystems. Tian et al. (2010) also found that C:P and N:P ratios showed high spatial heterogeneity and large variations in different climatic zones, soil orders, soil depths and weathering

stages. Furthermore, Zhang et al. (2016a); Zhang et al. (2016b) found that the bacterial relative abundance in a reclamation area was significantly lower than that in a non-reclamation area in the Yellow River Delta. The YNR in the Yellow River Delta supports a wide variety of flora and fauna, and more than 220 kinds of plant species and 800 kinds of animal species are found in this area (Cui et al., 2009). Plant N and P concentrations are strongly determined by the corresponding availabilities in soil, and plant litter serves as the primary C resource for soil decomposers. Plants encounter a wide range of elemental ratios, and their diversity are significantly influenced by soil nutrient levels, biota and stoichiometric requirements, and vice versa. It is suggested that the feedbacks from living organisms can modify soil nutrient contents and result in "Redfield-like" correlations between the elemental ratios of the biota and soil in terrestrial ecosystems (Cleveland and Liptzin, 2007; Sterner and Elser, 2002; Tian et al., 2010). Related studies found a wide range of elemental ratios among specific plants and plant groups (Qu et al., 2017; Velthuis et al., 2017; Zechmeister-Boltenstern et al., 2015; Zeng et al., 2016). We found that the R_{CP} showed relatively low variation due to sedimentation and was not remarkably consistent with the R_{CN} and R_{NP}, which showed high spatial heterogeneity and large variations in the 0–50 cm soil depth in the two parts of the YNR This finding confirms the need for the spatial heterogeneity in the soil, which tends to promote and maintain biodiversity in the YRD. Meanwhile, the value of R_{NP} in the 0–5 cm layer of OYD was notably higher than that of the CYD with a longer reclamation history. Finally, we suggested that the soil R_{NP} at different reclamation histories could be a good indicator of the artificial improvement status during soil development.

4.3. Nutrient limitation of the coastal estuary

There is a widespread belief that the prevalence of primary production is limited by N in terrestrial systems and by P in freshwater ecosystems (Elser et al., 2000b; Schlesinger and Bernhardt, 2013). Abundant data indicate that the growth and reproduction of autotrophs as well as large-scale ecosystem primary production are frequently limited by supplies of N or P in freshwater, marine and terrestrial environments (Ågren et al., 2012; Elser et al., 2007; Søndergaard et al., 2017). The coastal estuary, as a land and sea interaction zone, includes wetlands, freshwater, marine and terrestrial ecosystem types. N and P, as the key nutrient elements for predicting plant biomass, have also been massively altered by anthropogenic activities in coastal estuary areas. With a focus on the use of N and P limitations on the soil potential nutrients supplied for primary production, the paradigm that N is a major nutrient that limits primary production in coastal marine systems has been well established (Sundareshwar et al., 2003; Vitousek et al., 1997). Indeed, the content of N varied more greatly in OYD and CYD than the C and P contents (the c.v.s of N in OYD and CYD were 0.74 and 0.69, versus 0.32 and 0.30 for C and 0.13 and 0.17 for P), and R_{CP} presented a well-constrained ratio (C_{65}:P_1) for the entire soil profile in the YNR. Compared with inventory data sets on the national scale for China (Tian et al., 2010) and the global scale (Cleveland and Liptzin, 2007), we found that the mean R_{CN} values in the YNR (35.7 in OYD, and 43.9 in CYD) were much higher than those of China (11.9) and the world (14.3), the mean R_{NP} values (2.3 in OYD, and 2.0 in CYD) were much lower than those others (5.2 in China and 13.1 at the global scale). The mean R_{CP} values (65 in YNR) was at a similar level across China (61). These results indicate that N more easily becomes the limiting nutrient in soils and even the entire ecosystem in this coastal estuary. As a result, the R_{NP} (from 2.0 in CYD to 2.3 in OYD) was subtly altered by agricultural reclamation and fertilization.

5. Conclusions

Our work provides an extensive survey and assessment on the elemental stoichiometry (C, N, P) of soil in the YNR on a nature reserve scale. Despite the findings that the soil C, N and P contents and their stoichiometric ratios showed high spatial heterogeneity and large
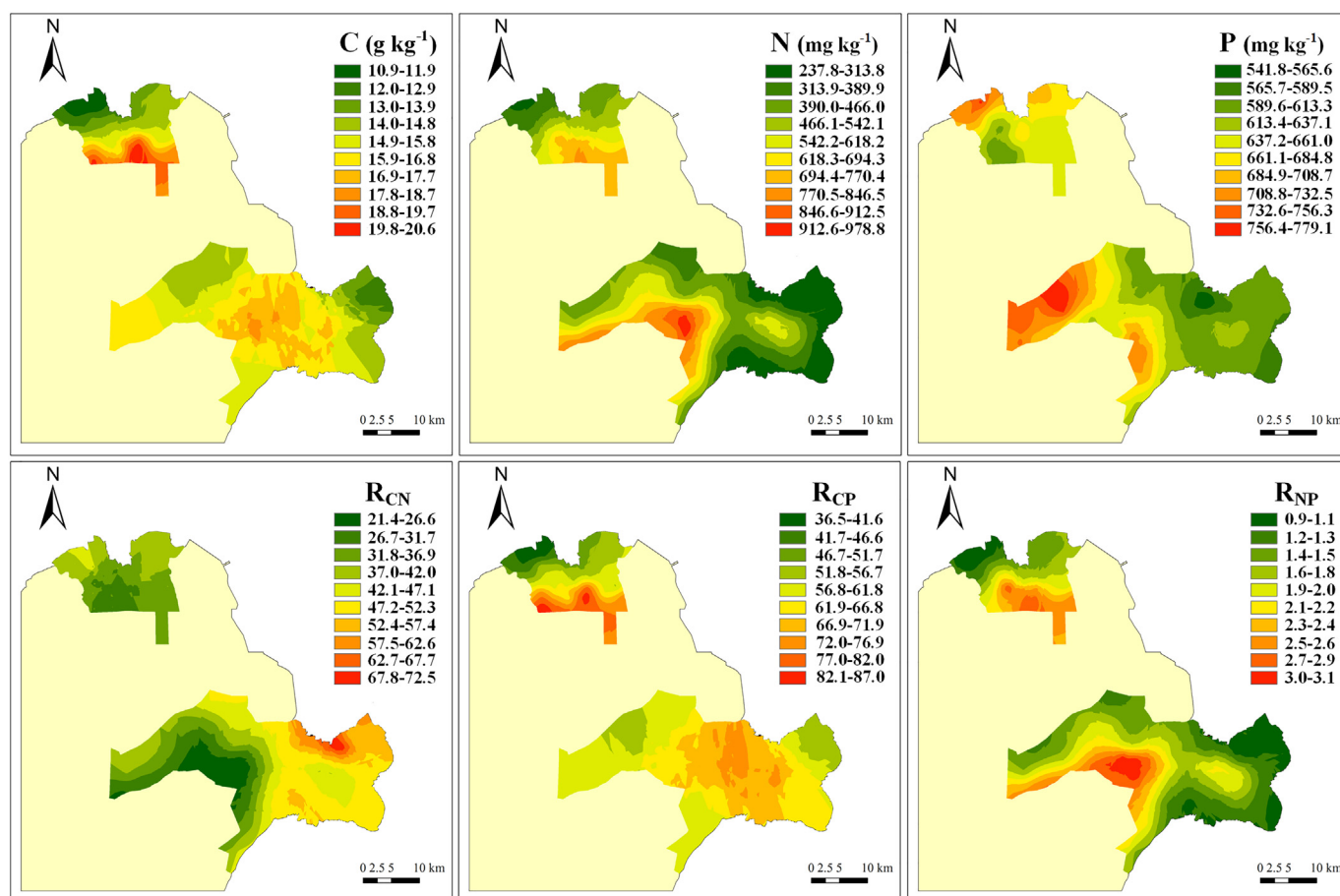
Fig. 4. Spatial distributions of soil C, N and P contents and their stoichiometric ratios in 0–50 cm soil depth in the YNR.

variations, we observed that a well-constrained R_{CP} ratio ~ 65:1 somewhat analogous to the Redfield ratio and the soil N limitation status in the YNR on a nature reserve scale, which indicated river sedimentation of the Yellow River played a major role in the contents and distributions of soil nutrients in this coastal estuary. R_{CN} in the 0–10 cm soil layers of OYD were significantly lower than that of CYD because of a longer reclamation and fertilization history leads to decreased R_{CN} ratios of coastal estuary soils. Considering that the soil R_{CP} ratios were primarily determined by the soil P content controlled by the sedimentation; and the soil R_{CN} and R_{NP} ratios were primarily influenced by N and P fertilization during reclamation, which can affect the soil development rate. We suggested that soil R_{CN} and R_{NP} could be good indicators of the anthropogenic improvement status during soil development in the coastal estuary. Consequently, our survey and assessment will provide a valuable reference for ongoing conservation and commercial exploitation of the coastal wetlands in the YRD.

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CRediT authorship contribution statement

Ling Meng: Data curation, Writing - original draft, Investigation, Methodology, Software, Formal analysis. **Fanzhu Qu:** Data curation,

Writing - original draft, Writing - review & editing, Project administration. **Xiaoli Bi:** Data curation, Writing - original draft, Writing - review & editing, Project administration. **Jiangbao Xia:** Investigation, Methodology, Software, Formal analysis, Writing - review & editing, Project administration. **Yunzhao Li:** Investigation, Methodology, Software, Formal analysis. **Xuehong Wang:** Investigation, Methodology, Software, Formal analysis. **Junbao Yu:** Writing - review & editing, Project administration.

Declaration of competing interest

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence our work reported in this paper.

Table 3
Summary of soil C, N and P stoichiometric ratios in the YNR.

Wetlands	n	R_{CN}	R_{CP}	R_{NP}	R_{CNP}
OYD	33	35.7 ± 15.9	64.7 ± 20.7	2.3 ± 1.6	64.7:2.3:1
CYD	87	43.9 ± 27.0	64.5 ± 18.0	2.0 ± 1.3	64.5:2.0:1

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