Increase soil aggregate stability can limit colloidal phosphorus loss potentials from agricultural systems

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Abstract

Background: Colloid-facilitated phosphorus (P) transport is a recognized important pathway for soil P loss in agricultural systems, but limited information is available on the soil aggregate-associated colloidal P. To elucidate the effects of aggregate size on the loss potential of colloidal P ($P_{\text{coll}}$) in agricultural systems, soils (0-20 cm depth) from six land use types were sampled in Zhejiang province in the Yangtz river delta region, China. The aggregate size fractions (2–8 mm, 0.26–2 mm, 0.053–0.26 mm and <0.053 mm) separated by wet-sieving method were analyzed.

Results: Results showed that the 0.26–2 mm small macroaggregates had the highest total P (TP) content. For acidic soils, the highest $P_{\text{coll}}$ content was also found in the 0.26–2 mm aggregate size, while the lowest was found in the <0.053 mm (silt+clay)-sized particles, the opposite of that found in alkaline soils. Paddy soils contained less $P_{\text{coll}}$ than other land use types. The $P_{\text{coll}}$ in total dissolved P (TDP) was dominated by <0.053 mm (silt+clay)-sized particles. Aggregate size did strongly influence the loss potential of $P_{\text{coll}}$ in paddy soils, where $P_{\text{coll}}$ contributed up to 83% TDP in the silt+clay sized particles. The $P_{\text{coll}}$ content was positively correlated with TP, Al, Fe and mean weight diameter (MWD). Aggregate associated total carbon (TC), total nitrogen (TN), C/P, and C/N had significant, but
negative effects on the contribution of $P_{\text{coll}}$ to potential soil P losses. The $P_{\text{coll}}$ content of the aggregates was controlled by aggregate associated TP and Al content as well as soil pH value, with $P_{\text{coll}}$ loss potential from aggregates being controlled by its organic matter content.

**Conclusion:** Therefore, we conclude that management practices that increase soil aggregate stability or its organic carbon content will limit $P_{\text{coll}}$ loss from agricultural systems.

**Keywords:** soil aggregate; colloidal phosphorus; pH; geometric mean diameter; loss of phosphorus.

**Background**

Phosphorus (P) loss from agricultural soils has been identified as one of the main causes of the eutrophication of lakes in the lower reaches of the Yangze River in southern China [1]. Soil colloidal phosphorus ($P_{\text{coll}}$) is the P fraction bound to colloids. Colloidal particles are highly mobile and are effective adsorbents for organic and inorganic contaminants and nutrient elements, such as P, because of high specific surface area and charge density, resulting in a high adsorption capacity [2, 3]. Colloid-facilitated P transport is an important pathway for the migration of P into water bodies [4-6]. Studies have shown that more than 75% of P in cultivated soil solution was combined with fine particles smaller than 240 nm [7];
similarly, 40–58% of molybdate-reactive P with size less than 450 nm in water extract of grassland soil was fine-grained P with size of 25–450 nm [5]. Other studies suggest that $P_{\text{coll}}$ can reach up to 50% of total P (TP) in surface runoff, rivers, and lakes [8], which may lead to eutrophication and environmental risks.

Water-dispersible colloids in the soil adhere to soil aggregates, forming a stable system [9]. Soil aggregate stability plays a key role in controlling erosion processes and soil nutrients losses [10-12]. Colloids either can bind to soil aggregates, or be physically strained from water flowing though pores between aggregates [13, 14]. Soil organic carbon (SOC) promotes aggregate stability and thus reduces the degree of clay colloid dispersion [15-17]. Some scholars determined the colloid content in 1-2 mm aggregates in 39 soils, and found a significant positively correlation between water dispersible colloid (WDC) content and clay content in soil aggregates [15]. Furthermore, they reported that, WDC content is a function of total organic C (TOC) and total clay. Other scholars modeled the release characteristics of colloids from soil aggregates, the attachment and detachment processes at the air-water interface, and flocculation and straining from interstitial water [18]. The release of colloids from aggregates may result in the disintegration of aggregates [19]. However, soil aggregation mainly depends on the
availability of active mineral surfaces and the dispersion/flocculation behaviors of the colloidal components [20]. Therefore, the stability of soil aggregates directly affects the migration of soil colloids.

The retention of P to soil aggregates depends on the particle size [21, 22]. Phosphorus has a relatively closed cycle in which most of the mineralized and dissolved P from the microaggregates is adsorbed onto unaggregated clay-sized particles (<53 μm) or is utilized by plants [23]. Some studies have shown that soil aggregate stability and size can affect soil P distribution [24-26]. Higher percentages of both water-extractable and Mehlich III-extractable P have been found in both the 0.50-0.25 and 0.25-0.125 mm aggregate fractions [27]. In contrast, some other researchers suggested that TP is highest in small soil aggregates [28], or the TP content was uniform in soil aggregates of all sizes, whereas the available P was higher in small soil aggregates [29]. Soil aggregation can significantly reduce the loss of organic P in aggregates and increase the adsorption of inorganic P by silt and clay particles [23]. Some scholars have studied different forms of P in soil aggregates. For example, a study showed that Al-P was mainly dominated by soil aggregates of <1 mm, while those of 2-8 mm were mainly Fe-P and Ca-P [30]. Others claimed that labile P in macroaggregates was higher under native land use than other land uses, they further confirmed that soils under native use
contained more Ca-bound P in macro-aggregates than the disturbed soils [31]. These studies provide a first basis for the better understanding the relationship between soil aggregates and P. To date, there is still limited information on $P_{\text{coll}}$ content and its loss potential from aggregates. It is unclear how aggregation affect the $P_{\text{coll}}$ content in soils. Moreover, the composition and structure of soil aggregates do vary under different land use managements [32]. There are less large-sized aggregates present in rice soil than dryland, which was due to the long-term flooding and anaerobic conditions that caused the macro-aggregates to be dispersed [32, 33]. In addition, alternation between dry and wet conditions generally destroys macro-aggregates and enhances the decomposition of SOC in paddy soils [34]. Therefore, we suspect that the content of $P_{\text{coll}}$ in macro-aggregates of paddy soils is less than dryland soils, but mainly exists in micro-aggregates and has a higher loss potential.

The main purposes of this study were to understand the effect of soil aggregate stability on soil $P_{\text{coll}}$ content and its loss potential, and to assess the main environmental factors affecting $P_{\text{coll}}$ in soil aggregates. To this end, we collected soil samples from 15 sites and 6 land use types in Zhejiang Province in the Yangtz river delta region for aggregates and $P_{\text{coll}}$ analysis. Firstly, we isolated the different sized aggregates in the soil samples. Secondly, we then determined the $P_{\text{coll}}$, total carbon (TC), TP,
total nitrogen (TN), Al, Fe and Ca content in each aggregate size fraction. We hypothesized that 1) Larger sized aggregates have higher TP and $P_{\text{coll}}$ content; 2) Aggregates with higher organic carbon content have lower loss potential of $P_{\text{coll}}$; and 3) Land use management with single rice has a higher loss potential of $P_{\text{coll}}$.

Materials and methods

Soil sampling and preparation

In total, soils with different land use types (Figure 1) were collected from 15 sites, which were almost evenly distributed in Zhejiang Province (an area of 1,055,000 km$^2$). Information on specific sampling points is shown in Table 1. The 15 sampling points covered six land use types, generally established in the past 5 years.

Soil samples of 0-20 cm were collected from typical fields (Long-term farmland with conventional fertilization by local farmers) in May 2018 in the second season of the rotation systems. Two samples with three replicates were taken at an interval of 1000 m in each site with same land use type, the replicates were brought back to the laboratory and mixed. Then, the mixed soil samples from each site were divided into four equal parts by the diagonal quartering method. One part was retained for a follow-up test. All samples were air-dried and separated into two parts;
one was finely milled and sieved through a 2-mm mesh to determine basic physical and chemical properties, and another was broken carefully into small pieces by hands to segregate aggregates and determine $P_{\text{coll}}$.

**Aggregate separation and determination**

Aggregate size distribution was determined for each soil sample using a modified wet sieving method [35]. Briefly, the second part of unground soil was first passed through an 8-mm sieve, and 50 g soil was placed carefully on the top of a nest of three sieves (2 mm, 0.26 mm, and 0.053 mm). Then, the sieves were submerged for 20 min in 2.5 L deionized water at room temperature and oscillated under water 300 times for 10 min with a 30 mm amplitude to separate aggregate fractions. Thus, four aggregates fractions were obtained on each sieve: large macroaggregates (2–8 mm), small macroaggregates (0.26–2 mm), microaggregate (0.053–0.26 mm), and (silt+clay)-sized particle (<0.053 mm) [36]. Aggregates of each size were carefully moved from the sieve into a beaker. The water used for wet sieving was left to rest for 48 h, silt and clay particles were collected, and the supernatant was used to determine total dissolved P (TDP), truly soluble P (TSP), and $P_{\text{coll}}$ content. All aggregates were oven-dried at 65 °C for 48 h, weighed, and placed in a zip lock bag. To obtain water-stable aggregates, sediment
concentration was subtracted from that obtained by wet sieving because sand was not considered a component of water-dispersible aggregates [35]. Sand content was determined by the following process: 5 g of the dry aggregates obtained above were weighed, dispersed into 30 mL 5 g L\(^{-1}\) hexametaphosphate solution, placed into an ultrasonic cleaner, and dispersed for 30 min. The suspension was then poured through a nest of sieves. The residue left on the 0.053 mm sieve represented the sand content of each sized aggregate. After collection, sand was dried at 65 °C and weighed.

The pH of soils was determined with a glass electrode pH meter (PHS-3C, Shanghai) at a soil-to-water ratio of 1:5. Soil cation exchange capacity (CEC) was measured with ammonium acetate (12.5 mL 1M NH\(_4\)OAc, 2.5 g soil) [37]. Soil and aggregates associated TP was determined by H\(_2\)SO\(_4\)-HClO\(_4\) digestion and evaluated by the molybdenum-blue colorimetric method [38]. Soil particle size distribution was determined by the hydrometric method according to an international soil texture classification standard. Soil- and aggregate-associated TC and TN were determined with an elemental analyzer (dry combustion with Vario MAX CNS, Elementar, Germany). Soil- and aggregate-associated Al, Fe, and Ca were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after digestion with 5 mL HNO\(_3\) (16
M), 1 mL HClO₄ (12.4 M), and 1 mL HF (23 M) for 12h. All reagents were from Sinopharm Chemical Reagent Co., Ltd.

Colloidal P was determined as described by Ilg [39]. Briefly, 10 g of unground soil was placed into a 250 mL flask, 80 mL deionized water (DDW) was added, and the sample was shaken at 160 rpm and 25°C for 24 h. The supernatant was pre-centrifuged at 3000 g for 10 min to remove coarse particles. After pre-centrifugation, the supernatant was filtered with a 1 μm microporous membrane, 5 mL of the primary filtrate was discarded, and the total filtrate was collected (sample I). This suspension included the colloidal component and the dissolved component. The filtrate was ultracentrifuged at 300,000 g for 2 h to remove colloids (Optima TL, Beckman, USA; Sample II), and the residue at the bottom of the ultracentrifuge tube was the water-dispersible colloid. The TDP in sample I and TSP in sample II in the solution were determined after digestion with acidic potassium persulfate. The difference between TDP in Sample I and TSP in Sample II was the concentration of $P_{\text{coll}}$. Previous studies have shown that soil P through leaching and surface runoff is usually in soluble forms that can pass through the 0.45–1 μm filter [40, 41]; therefore, in the present study, TDP including $P_{\text{coll}}$ and TSP in aggregates was defined as the potential loss P. The TDP in the supernatant after 10 min wet sieving was
Considered as easy loss P.

Calculation of water-stable aggregate (WSA) size fractions

The proportion of WSA in each size fraction was obtained from equations (1) and (2), as described by Alvaro-Fuentes [42]:

\[
WSA_i = \frac{DSA_i - Sand_i}{Total_{Soil} - \sum_{i=1}^{n} Sand_i}
\]  
(1)

\[(silt + clay) sized fraction = 1 - \sum_{i=1}^{n} WSA_i \]  
(2)

where, i is the ith size fraction (2–8, 0.25–2, and 0.053–0.25 mm); dry soil aggregate (DSA_i) is the oven-dried mass of total, non-dispersed aggregates collected on each sieve; Sand is the oven-dry mass of sand collected after dispersed in hexametaphosphate solution on the 0.053 mm sieve; Total_{Soil} is the oven-dried mass of soil (50 g) for aggregate separation.

Calculation of MWD and GMD

The mean weight diameter (MWD) and geometric mean diameter (GMD) of the aggregates can be obtained by Equations (3) and (4) [43]:

\[
MWD = \sum_{i=1}^{n} d_i \times WSA_i
\]  
(3)

\[
GMD = \exp \left[ \frac{\sum_{i=1}^{n} WSA_i \log d_i}{\sum_{i=1}^{n} WSA_i} \right]
\]  
(4)

where, i is the ith size fraction (2–8, 0.26–2, 0.053–0.26, and <0.053 mm) and d is the mean diameter of each size (0.053–2 mm). WSA_i include 2–8,
0.26–2, 0.053–0.25, and <0.053 mm WSA and (silt+clay) sized fractions.

**Contribution of aggregate associated $P_{\text{coll}}$ to TDP**

To illustrate the contribution of aggregate associated $P_{\text{coll}}$ to TDP (potential loss $P_{\text{p}}$), soil aggregates and $P_{\text{coll}}$ concentrations were integrated and calculated. The contribution rate (CR) of aggregate associated $P_{\text{coll}}$ to TDP was calculated using Equation (5):

$$CR_i = \frac{\text{Agg}_i \times WSA_i}{\sum_{i=1}^{n} TDP}$$

where, $\text{Agg}_i$ is the concentration of aggregate associated $P_{\text{coll}}$ (mg kg$^{-1}$), TDP is the concentration total dissolved $P$ (mg kg$^{-1}$), and $i$ is the ith size fraction (2–8, 0.26–2, 0.053–0.26, and <0.053 mm).

**Statistical analysis**

Microsoft Excel 2016 and Origin 8.0 were used for data processing and cartography. Data were statistically analyzed using SPSS Statistics 22.0 (SPSS Inc. Chicago, USA) software. One-way ANOVA was conducted using two samples of each site to examine differences of different variables in Table S1, S2, and 3 and Fig S1 and S2. Pearson correlation analyses were used to identify the relationship between aggregate associated $P_{\text{coll}}$ and other soil parameters. Stepwise linear regression was performed to evaluate the relationships between $P$ indicators (Content...
and loss potential of P$_{coll}$ and soil variables (pH, TP, TC, TN, C/N, Fe, Al, Ca, MWD, and GMD).

Results

Soil and aggregate characteristics

Within the collected 15 soils, ten were acidic soils, two were neutral soils, and three were alkaline soils (Table 1). There were six land use types: orchard, single cropping rice, rice-rape rotation, vegetable, double cropping rice, and rice-wheat rotation. The TC of the soils ranged from 3.32 to 20.19 g kg$^{-1}$; the TN was between 0.53 and 2.17 g kg$^{-1}$. The TP varied from 0.23 to 1.64 g kg$^{-1}$. Soil pH values ranged from 3.95 to 7.83 (Table 1).

The DSA and WSA of larger macroaggregates (2–8 mm) generally increased with increasing of pH values (Figure S1 and Figure S2, Table S2). Moreover, fractions of DSA and WSA (2–8 mm) in acidic soils ranged from 18.7 to 33.4% and from 10.5 to 47.1%, respectively. Conversely, fractions of DSA and WSA in alkaline soils ranged from 39.7 to 56.1% and from 38.1 to 55.6%, respectively. In contrast, the size fractions of < 0.053 mm particles gradually decreased with increasing soil pH values, while the average contents of < 0.053 mm particles in acidic soils were 18.8 and 17.8%, compare with only 11.2% and 14.7% in alkaline soils,
respectively.

The MWDs of acidic soils (pH < 5.5) were significantly smaller than those of alkaline soils ($P < 0.05$). The average MWD of acidic soils was 0.78 mm (Figure S2), while that of neutral and alkaline soils was 1.36 mm. However, little difference in GMDs was found between acidic (0.85 mm), and alkaline soils (0.91 mm).

**Total and colloidal phosphorus content**

Generally, the 0.26–2 mm aggregate fraction had the highest TP content, which accounted for 29.6% of the soil TP (Fig.1a), and soil aggregates of 2–8 mm had the second highest TP content. Whilst that of (silt+clay) sized particles was significantly lower than that of other fractions ($P < 0.05$, Table S1), which only accounted for 19.7% of the soil TP (Fig.1a). Moreover, TDP, TSP, and P$_{coll}$ contents were related to soil pH, and the highest TDP content was found in 0.26–2 mm aggregates in acidic soils and in (silt+clay) sized particles in alkaline soils ($P < 0.05$, Table S1). In all soils, no significant difference was observed in the P$_{coll}$ fractions between different aggregate sizes ($P > 0.05$; Fig. 1b). However, the content of aggregate associated P$_{coll}$ was highest in the 0.26–2mm aggregates, and the lowest in (silt+clay)-sized particles in acidic soils; while in neutral and alkaline soils, (silt+clay)-sized particles possessed
the highest TDP and $P_{\text{coll}}$ contents, followed by the 0.26–2 mm aggregates (Table 3).

**Loss potential of colloidal phosphorus**

After wet sieving, about 0.16–1.87% of the soil TP was lost into the supernatant as TDP, and the $P_{\text{coll}}$ accounts for 8.5%–84.1% of the TDP (Table 2). The proportion of easy loss P content in the various soils was also different due to variations in soil physicochemical properties.

To make the $P_{\text{coll}}$ in different soils comparable, the indicator of $P_{\text{coll}}$ in TDP was used for standardization. The proportion of aggregate-associated $P_{\text{coll}}$ in TDP can reflect the loss potential of $P_{\text{coll}}$ in the TDP. In general, the $P_{\text{coll}}$ loss potential gradually decreases as the size of soil aggregates increases. The $P_{\text{coll}}$ loss potential was the lowest in 2–8 mm and 0.26–2 mm aggregates, and $P_{\text{coll}}$ accounted for 52.6% and 60.6% of TDP, respectively. However, the $P_{\text{coll}}$ loss potential of (silt+clay)-sized particles was the highest, and $P_{\text{coll}}$ accounted for 75.3% of TDP (Fig. 1a). The CR value of (silt+clay)-sized particles were mostly larger than that of the other aggregate sizes except for S7, S11 and S15, while the CR of larger macroaggregate was lowest in most soils (Table 3).

Considering different land use types, the $P_{\text{coll}}$ content in the rice-dry land rotation and vegetable (VE) soils was significantly higher than that
in paddy (RICE) and orchard (OR) soils regardless of different aggregate sizes (Figure 4a). Higher $P_{\text{coll}}$ content also was found in macroaggregates (> 0.26 mm) except for the orchard soil (Figure 4a). Colloidal P content in macro-and microaggregates in the paddy soils (including single cropping rice, and double cropping rice) accounted for a relatively lower proportion of TDP ($P < 0.05$) than that in other land use types (Figure 4b). Overall, it which only accounted for 39.3, 44.1, and 40.2% for the 2–8 mm, 0.26–2, and 0.053–0.26 mm aggregates in rice fields, respectively. While $P_{\text{coll}}$ in (silt+clay)-sized particles (<0.053 mm) accounted for the proportion of TDP were as high as 83.0% and all significantly higher than that in orchard and rice-dryland rotation systems (Figure 4b). This indicates that the loss potential of $P_{\text{coll}}$ was dominated by fine grained and (silt+clay) sized particles in paddy soils. However, the loss potential of $P_{\text{coll}}$ carried by all sized aggregates in dryland and rice-dryland rotation systems (orchard, rice-rape rotation, vegetable, and rice-wheat rotation) exceeded 50% of TDP.

**Factors affecting colloidal P content and loss potential**

Correlation analysis revealed that no significant correlations were found between $P_{\text{coll}}$ and TC or TN (Table 4). The $P_{\text{coll}}$ content was significantly positively correlated with TP in all aggregate sizes except for
(silt+clay)-sized particles (Fig 3a). Soil pH was positively correlated with the aggregate-associated P\text{coll} content, but was only significantly correlated with the P\text{coll} content of (silt+clay)-sized particles (\(P < 0.01\)) (Fig 3b). Al and Fe were also found significantly positively correlated with P\text{coll} in total sizes of soil aggregate (\(P < 0.05\)) (Fig 3c and d), however, only P\text{coll} in 2–8 mm aggregate were found significantly positively correlated with Al and Fe (\(P < 0.05\)). Moreover, P\text{coll} was negatively correlated with C/P (Fig 3e) and positively correlated with C/N (Fig 3f). The forward results of the stepwise regression showed that P\text{coll} can be described by Al, TP, TN and MWD as:

\[
\text{Colloidal P} = -8.017 + 0.087\text{Al} + 10.598\text{TP} - 2.257\text{pH} - 5.977\text{MWD}, \quad (R^2=0.605, \quad P < 0.001, \quad \text{Table 4}).
\]

Table 5 shows that the TC, TN, pH, Ca, C/P, C/N ratios of aggregates were negatively correlated with the proportion of P\text{coll} in TDP in total sizes of soil aggregate (Table 4, \(P < 0.05\)). Considering different sizes, TC and TN were only found significantly negatively correlated with P\text{coll} in 0.26–2 mm sized aggregates. Except for (silt+clay)-sized particles, Al was significantly positively correlation with P\text{coll} in TDP (\(P < 0.05\)). While Fe was significantly positively correlation with P\text{coll} in TDP in 2–8 mm sized aggregates (\(P < 0.05\)). However, Ca was negatively correlated with P\text{coll} in TDP in 0.053–2 mm sized aggregates (\(P < 0.05\)). The results of the
stepwise regression showed that $P_{coll}/TDP$ can be described by TC, Fe, MWD, and GMD as:

$$\text{Colloidal P/TDP} = 0.478 - 0.028\text{TC} + 0.015\text{Fe} + 0.413\text{MWD} - 0.363\text{GMD}, \quad (R^2=0.539, \quad P < 0.001, \text{Table 5})\). $

Discussions

Total P in aggregates

In our study, we found that the TP content was highest in small macroaggregates of the 15 soils, while the TP content in the silt+clay particles was the lowest. These results indicated that soil P was mainly carried by larger aggregates, which confirmed our hypothesis and were in line with those of previous studies [25, 44, 45]. For examples, some scholars found that P tended to concentrate in large WSAs in long-term fertilization experiments in a reddish paddy soil [44], and others claimed that aggregate-associated TOC, TN, and TP were preferentially enriched in large WSAs (4.76–2.0 mm) [45], and found that the proportion of TP increased with increasing aggregate size for native lands [31]. Higher P levels may be associated with higher levels of TC and TN in large aggregates [45, 46]. Studies have shown that the content of organic and inorganic P in 2–4 mm aggregates was higher [47]. Macroaggregates ([(Cl–P–OM)x]y) are usually formed by organic matter (OM), clay (Cl) and
multivalent ions of P and other substances [48, 49]. Organic matter (and associated P) are protected within stable aggregates against microbial degradation [50]. Moreover, it has been shown that the organic P forms that accumulate in soils are less available to enzymatic hydrolysis when bound to mineral surfaces [51, 52]. On the other hand, the aggregation promoted by the organic matter counteracts the dispersion of the small mineral particles (mostly Fe and Al (hydr) oxides) where P is retained [53, 54]. This is also evidenced by the positive correlation between aggregate-associated TP and TC, and the significant correlation between aggregate-associated TP and TN observed in this study (Tables S2).

**Colloidal P content in aggregates**

In this study, we found that TDP, TSP, and \( P_{coll} \) contents were related to soil pH. The TDP and \( P_{coll} \) contents were high in large aggregates of acidic soils, and low in micro-aggregates and silt- and clay-sized particles; however, contrasting results were found in alkaline soil aggregates. This is not consistent with our hypothesis. First this may be due to the acidity of the soil leading to the dissolution of organic matter and inorganic cement (\( Al^{3+}, Fe^{2+}, \) and \( Ca^{2+} \)) in the aggregates, causing the macroaggregates to be extremely unstable [55]. Moreover, soil clays are mostly negatively charged, but the aggregation of another soil colloids
strongly depends on their surface charge, being favored when
approaching their point of zero charge [56]. Protonation of Fe-, Al-oxides
and organic matter in colloids under acidic conditions results in positive
charges, leading to their association with soil particles. However, the
dissociation of Fe- and Al-hydroxyl and humic functional groups (R-COOH,
R-\(\text{CH}_2\-\text{OH}\), R-OH) under alkaline conditions results in a negative charge
of the colloid [56], which promote the release of fine particulate P and
colloidal substances, thus increasing the TDP and \(P_{\text{coll}}\) contents in small
sized aggregate and particles.

In addition, we found that the \(P_{\text{coll}}\) content in soil aggregates was
positively correlated with the aggregate-associated Al and Fe content.
This is because the contents of Fe and Al in acidic soil are greater than
that in alkaline soil and the presence of Al and Fe oxides may have
enhanced the adsorption of P and stabilization of soil aggregates [9, 28,
57, 58]. Al and Fe oxides also had been recognized as important carriers
of \(P_{\text{col}}\) [59-62].

We also found that the CR value of (silt+clay) sized particles was
larger than that of aggregates of other sizes, while the CR of large
macroaggregates was the lowest in most soils, which also indicates that
the (silt+clay) sized particles contribute more for the \(P_{\text{Coll}}\) loss potential,
while macroaggregates immobilized the soil \(P_{\text{coll}}\). Colloidal P is highly
bonded to Fe and Al on the surface of the macroaggregates [9, 63], making it easy to form a stable composite structure that can resist the shear force of pore flow [59, 60]. Similarly, soil macroaggregates also can increase the adsorption of $P_{\text{coll}}$ on the surface and reduce its mobility [23]. The ratio of $P_{\text{coll}}$ to TP in soil aggregates was not related to soil TP content (Table S2), indicating that the aggregate associated TP content had no significant effect on the proportion of $P_{\text{coll}}$ in TP.

**Loss potential of colloidal P in aggregates**

The ratio of $P_{\text{coll}}$ to TDP reflects the release potential of $P_{\text{coll}}$ in soil aggregates to soil solution. The high value indicates the high loss potential of $P_{\text{coll}}$. We observed a negative correlation between TC and TN content and $P_{\text{coll}}$/TDP in aggregates indicating that the higher the TC and TN content, the less likely release of the $P_{\text{coll}}$ from soil aggregates, which is also confirmed by the negative correlation between TC and $P_{\text{coll}}$/TDP in the regression model (Table 4). Studies had showed that C and N are important carriers of $P_{\text{coll}}$ (Most organics act as organic colloidal complexes) [25, 64, 65], and the organic matter would stabilize Al/Fe colloids [66]. Therefore, increasing the carbon content in soil aggregates may be an important strategy to reduce the migration of $P_{\text{coll}}$ in soil. The ratio of $P_{\text{coll}}$ to TDP for all macroaggregates in paddy soils was lower,
which may be related to the long-term flooding of rice fields. Notably, the
lower loss potential of $P_{\text{coll}}$ in macroaggregates of paddy soils does not
mean it is unlikely to be lost to water bodies, however, it may indicate
that the $P_{\text{coll}}$ has been lost into water and discharged into the water body
through the channels during rice seasons when under long-term flooding
[67]. Moreover, flooding resulted in an anaerobic state in soil aggregates,
and Fe bound to colloids and aggregates was reduced, resulting in
excessive release and loss of $P_{\text{coll}}$ [9]. We found that aggregates of all
sizes in dryland and rice-dry rotation systems carried higher $P_{\text{coll}}$ loss
potential, and the loss potential of $P_{\text{coll}}$ carried by large-grained soil
aggregates in paddy soil was lower than 50% of TDP. However, $P_{\text{coll}}$ in
(silt+clay)-sized particles (<0.053 mm) accounted for the proportion of
TDP was as high as 83% and significantly higher than that in in orchard
and rice-dryland rotation systems. Therefore, we believe that $P_{\text{coll}}$ in
paddy soil is mainly carried by small silt and clay particles, which may
indicate that the loss of $P_{\text{coll}}$ is remarkable severe in paddy soils.

Conclusions

We found that the aggregate size distribution of soil $P_{\text{coll}}$ was positively
affected by pH values, and the highest $P_{\text{coll}}$ content of small
macroaggregates occurred in acidic soils. Small macroaggregates
contributed the most to the immobilization of $P_{\text{coll}}$. The soil $P_{\text{coll}}$ content was affected by multiple factors including soil Al, Fe, and TP. However, soil $P_{\text{coll}}$ loss was primarily associated with aggregate stability and negatively associated with OM for any given soil type. Paddy soils, because of their high $P_{\text{coll}}$/TDP ratio, are particularly at risk of high $P_{\text{coll}}$ loss by smaller particles. Therefore, management practices that increasing soil aggregate stability or its organic carbon content will help to control and limit $P_{\text{coll}}$ and thus also overall P loss from agricultural systems.

**Abbreviations**

P: phosphorus; $P_{\text{coll}}$: colloidal phosphorus; TP: total phosphorus; SOC: soil organic carbon; WDC: water dispersible colloid; TOC: total organic C; TC: total carbon; TN: total nitrogen; TDP: total dissolved P; TSP: truly soluble P; CEC: cation exchange capacity; DDW: deionized water; WSA: water-stable aggregate; DSA: MWD: dry soil aggregate; mean weight diameter; GMD: geometric mean diameter; CR: contribution rate; OM: organic matter; OR: orchard soils; RICE: Rice fields including single and double cropping soils; RDR: Rice and dry land rotation including rice-rape rotation and rice-wheat rotation soils; VE: vegetable soils.
Declarations:

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare no competing interests.

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Author Contributions
FYL is the first author of this article, XQL is the corresponding author,
FYL, MMH, YBJ, and JJW designed this study and conducted data analysis, XQL and HL provide financial support in the form of this project. EK and RB provided specific guidance on this research and revised this MS. FYL wrote the manuscript and had primary responsibility for the final content. All authors read and approved the final manuscript.

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References

1. Le C, Zha Y, Li Y, Sun D, Lu H, Yin B (2010) Eutrophication of lake waters in China: cost, causes, and control. Environ Manage 45:662– 668. https://doi:10.1007/s00267-010-9440-3

2. Kretzschmar R BM, Grolimund D, Elimelech M (1999) Mobile subsurface colloids and their role in contaminant transport. Adv Agron 66:121–193. https://doi:10.1016/s0065-2113(08)60427-7
3. Norgaard T, Moldrup P, Ferre TP, Katuwal S, Olsen P, de Jonge LW (2014) Field-scale Variation in Colloid Dispersibility and Transport: Multiple Linear Regressions to Soil Physico-Chemical and Structural Properties. J Environ Qual 43:1764–1778. https://doi:10.2134/jeq2013.12.0510

4. Heathwaite L, Haygarth P, Matthews R, Preedy N, Butler P (2005) Evaluating colloidal phosphorus delivery to surface waters from diffuse agricultural sources. J Environ Qual 34:287–298. https://doi:10.2147/OTT.S71242

5. Hens M, Merckx R (2001) Functional characterization of colloidal phosphorus species in the soil solution of sandy soils. Environ Sci Technol 35:493-500. https://doi:10.1021/es0013576

6. Liang X, Jin Y, Zhao Y, Wang Z, Yin R, Tian G (2016) Release and migration of colloidal phosphorus from a typical agricultural field under long-term phosphorus fertilization in southeastern China. J Soils Sediments 16:842–853. https://doi:10.1007/s11368-015-1290-4

7. de Jonge LW, Kjærgaard C, Moldrup P (2004) Colloids and colloid-facilitated transport of contaminants in soils. Vadose Zone J 3:321–325. https://doi:10.2136/vzj2004.0321

8. Mayer T, Jarrell W (1995) Assessing colloidal forms of phosphorus and
iron in the Tualatin River Basin. J Environ Qual 24:1117–1124. https://doi:10.2134/jeq1995.00472425002400060010x

9. Henderson R, Kabengi N, Mantripragada N, Cabrera M, Hassan S, Thompson A (2012) Anoxia-induced release of colloid- and nanoparticle-bound phosphorus in grassland soils. Environ Sci Technol 46:11727–11734. https://doi:10.1021/es302395r

10. Shi P, Arter C, Liu X, Keller M, Schulin R (2017) Soil aggregate stability and size-selective sediment transport with surface runoff as affected by organic residue amendment. Sci Total Environ 607:95-102. https://doi:10.1016/j.scitotenv.2017.07.008

11. Whalen JK, Chang C (2002) Macroaggregate characteristics in cultivated soils after 25 annual manure applications. Soil Sci Soc Am J 66:1637–1647. https://doi:10.1007/s10705-007-9130-6

12. Wortmann C, Shapiro C (2008) The effects of manure application on soil aggregation. Nutr Cycling Agroecosyst 80:173–180. https://doi:10.1007/s10705-007-9130-6

13. Bradford SA, Yates SR, Bettahar M, Simunek J (2002) Physical factors affecting the transport and fate of colloids in saturated porous media. Water Resour Res 38: 63-1–63-12. https://doi:10.1029/2002WR001340

14. DeNovio NM, Saiers JE, Ryan JN (2004) Colloid movement in
unsaturated porous media. Vadose Zone J 3:338–351. https://doi:10.2136/vzj2004.0338

15. Lægdsmand M, de Jonge LW, Moldrup P (2005) Leaching of colloids and dissolved organic matter from columns packed with natural soil aggregates. Soil Sci 170:13–27. https://doi:10.1097/00010694-200501000-00003

16. Lekfeldt JDS, Kjaergaard C, Magid J (2017) Long-term effects of organic waste fertilizers on soil structure, tracer transport, and leaching of colloids. J Environ Qual 46:862–870. https://doi:10.2134/jeq2016.11.0457

17. Munkholm LJ, Schjønning P, Deboz K, Jensen HE, Christensen BT (2002) Aggregate strength and mechanical behaviour of a sandy loam soil under long-term fertilization treatments. Eur J Soil Sci 53:129–137. https://doi:10.1046/j.1365-2389.2002.00424.x

18. Lægdsmand M, Moldrup P, De Jonge LW (2007) Modelling of colloid leaching from unsaturated, aggregated soil. Eur J Soil Sci 58:692–703. https://doi:10.1111/j.1365-2389.2006.00854.x

19. Vendelboe AL, Schjønning P, Moldrup P, Jin Y, Merbach I, de Jonge LW (2012) Colloid release from differently managed loess soil. Soil Sci 177:301–309. https://doi:10.1097/ss.0b013e3182506dd3

20. Vogel C, Mueller CW, Hoschen C, Buegger F, Heister K, Schulz S,
Schloter M, Kogel-Knabner I (2014) Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. Nat Commun 5:2947. https://doi:10.1038/ncomms3947

21. Ranatunga TD, Reddy SS, Taylor RW (2013) Phosphorus distribution in soil aggregate size fractions in a poultry litter applied soil and potential environmental impacts. Geoderma 192:446-452. https://doi:10.1016/j.geoderma.2012.08.026

22. Hao X, Chang C, Li X (2004) Long-term and residual effects of cattle manure application on distribution of P in soil aggregates. Soil Sci 169:715-728. https://doi:10.1002/jpln.200320355

23. Garland G, Bünemann E, Oberson A, Frossard E, Snapp S, Chikowo R, Six J, Biochemistry (2018) Phosphorus cycling within soil aggregate fractions of a highly weathered tropical soil: A conceptual model. Soil Biol Biochem 116:91-98. https://doi:10.1016/j.soilbio.2017.10.007

24. Udom BE, Ogunwole JO (2015) Soil organic carbon, nitrogen, and phosphorus distribution in stable aggregates of an Ultisol under contrasting land use and management history. J Plant Nutr Soil Sci 178:460-467. https://doi:10.1002/jpln.201400535

25. Jiang X, Bol R, Willbold S, Vereecken H, Klumpp E (2015) Speciation and distribution of P associated with Fe and Al oxides in aggregate-sized fraction of an arable soil. Biogeosciences 12:6443-
26. Cui H, Ou Y, Wang L, Wu H, Yan B, Li Y (2019) Distribution and release of phosphorus fractions associated with soil aggregate structure in restored wetlands. Chemosphere 223:319–329. https://doi:10.1016/j.chemosphere.2019.02.046

27. Zhang M, He Z, Calvert D, Stoffella P, Yang X, Li Y (2003) Phosphorus and heavy metal attachment and release in sandy soil aggregate fractions. Soil Sci Soc Am J 67:1158–1167. https://doi:10.2136/sssaj2003.1158

28. Wang JG, Yang W, Yu B, Li ZX, Cai CF, Ma RM (2016) Estimating the influence of related soil properties on macro-and micro-aggregate stability in ultisols of south-central China. Catena 137:545–553. https://doi:10.1016/j.catena.2015.11.001

29. Rubæk GH, Guggenberger G, Zech W, Christensen BT (1999) Organic phosphorus in soil size separates characterized by phosphorus-31 nuclear magnetic resonance and resin extraction. Soil Sci Soc Am J 63:1123–1132. https://doi:10.2136/sssaj1999.6351123x

30. Wu W, Zheng Z, Li T, He S, Zhang X, Wang Y, Liu T, sediments (2018) Distribution of inorganic phosphorus fractions in water-stable aggregates of soil from tea plantations converted from farmland in the hilly region of western Sichuan, China. J soil 18:906–916.
31. Sheklabadi M, Mahmoudzadeh H, Mahboubi A, Gharabaghi B, Ahrens B, assessment (2014) Land use effects on phosphorus sequestration in soil aggregates in western Iran. Environ Monit 186:6493–6503. https://doi:10.1007/s10661-014-3869-4

32. Huang R, Lan M, Liu J, Gao M (2017) Soil aggregate and organic carbon distribution at dry land soil and paddy soil: the role of different straws returning. Environ Sci Pollut Res 24:27942–27952. https://doi:10.1007/s11356-017-0372-9

33. Li S, Zhang S, Pu Y, Li T, Xu X, Jia Y, Deng O, Gong G (2016) Dynamics of soil labile organic carbon fractions and C-cycle enzyme activities under straw mulch in Chengdu Plain. Soil Tillage Res 155:289–297. https://doi:10.1016/j.still.2015.07.019

34. Wang X, Qi JY, Zhang XZ, Li SS, Virk AL, Zhao X, Xiao XP, Zhang HL (2019) Effects of tillage and residue management on soil aggregates and associated carbon storage in a double paddy cropping system. Soil Tillage Res 194:104339. https://doi:10.1016/j.still.2019.104339

35. Elliott ET (1986) Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils 1. Soil Sci Soc Am J 50:627–633. https://doi:10.2136/sssaj1986.03615995005000030017x

36. Totsche KU, Amelung W, Gerzabek MH, Guggenberger G, Klumpp E,
Knief C, Lehndorff E, Mikutta R, Peth S, Prechtel A (2018) Microaggregates in soils. J Plant Nutr Soil Sci. 181:104–136. https://doi:10.1002/jpln.201600451

37. Sumner ME, Miller WP (1996) Cation exchange capacity and exchange coefficients. Methods of soil analysis part 3—chemical methods 1201–1229. https://doi:10.1097/00010694-197006000-00009

38. Walker T, Adams A (1958) Studies on soil organic matter: I. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. Soil Sci 85:307–318. https://doi:10.1097/00010694-195901000-00001

39. Ilg K, Siemens J, Kaupenjohann M (2005) Colloidal and dissolved phosphorus in sandy soils as affected by phosphorus saturation. J Environ Qual 34:926–935.

40. Beauchemin S, Simard R, Cluis D (1998) Forms and concentration of phosphorus in drainage water of twenty-seven tile-drained soils. J Environ Qual 27:721–728. https://doi:10.2134/jeq1998.00472425002700030033x

41. Beauchemin S, Simard R, Bolinder M, Nolin M, Cluis D (2003) Prediction of phosphorus concentration in tile-drainage water from the Montreal Lowlands soils. Can J Soil Sci 83:73–87. https://doi:10.4141/S02-029
42. Álvaro-Fuentes J, Cantero-Martínez C, López M, Paustian K, Denef K, Stewart C, Arrúe J (2009) Soil aggregation and soil organic carbon stabilization: effects of management in semiarid Mediterranean agroecosystems. Soil Sci Soc Am J 73:1519–1529. https://doi:10.2136/sssaj2008.0333

43. Van Bavel C (1950) Mean weight-diameter of soil aggregates as a statistical index of aggregation 1. Soil Sci Soc Am J 14:20–23. https://doi:10.2136/sssaj1950.036159950014000C0005x

44. Wang W, Chen WC, Wang KR, Xie XL, Yin CM, Chen AL (2011) Effects of long-term fertilization on the distribution of carbon, nitrogen and phosphorus in water-stable aggregates in paddy soil. Agric Sci China 10:1932–1940. https://doi:10.1016/S1671-2927(11)60194-6

45. Adesodun J, Adeyemi E, Oyegoke C (2007) Distribution of nutrient elements within water-stable aggregates of two tropical agro-ecological soils under different land uses. Soil Tillage Res 92:190–197. https://doi:10.1016/j.still.2006.03.003

46. Wang S, Li T, Zheng Z (2018) Tea plantation age effects on soil aggregate-associated carbon and nitrogen in the hilly region of western Sichuan, China. Soil Tillage Res 180:91–98. https://doi:10.1016/j.still.2018.02.016
47. Russell A, Kivlin S, Hawkes C (2018) Tropical tree species effects on soil pH and biotic factors and the consequences for macroaggregate dynamics. Forests 9:184. https://doi:10.3390/f9040184

48. Edwards A, Bremner J (1967) Microaggregates in soils 1. J Soil Sci 18:64–73. https://doi:10.1111/j.1365-2389.1967.tb01488.x

49. Bronick CJ, Lal R (2005) Soil structure and management: a review. Geoderma 124:3–22. https://doi:10.1016/j.geoderma.2004.03.005

50. Awad YM, Lee SS, Kim KH, Ok YS, Kuzyakov Y (2018) Carbon and nitrogen mineralization and enzyme activities in soil aggregate-size classes: Effects of biochar, oyster shells, and polymers. Chemosphere 198:40–48. https://doi:10.1016/j.chemosphere.2018.01.034

51. Giaveno C, Celi L, Richardson AE, Simpson RJ, Barberis E (2010) Interaction of phytases with minerals and availability of substrate affect the hydrolysis of inositol phosphates. Soil Biol Biochem 42:491–498. https://doi:10.1016/j.soilbio.2009.12.002

52. George TS, Simpson RJ, Gregory PJ, Richardson AE (2007) Differential interaction of Aspergillus niger and Peniophora lycii phytases with soil particles affects the hydrolysis of inositol phosphates. Soil Biol Biochem 39:793–803. https://doi:10.1016/j.soilbio.2006.09.029

53. Yang Y, Liu S, Wang H, Chen L, Lu L, Cai D (2019) Reduction in
throughfall reduces soil aggregate stability in two subtropical plantations. Eur J Soil Sci 70:301–310. https://doi:10.1111/ejss.12734

54. He Y, Gu F, Xu C, Chen J (2019) Influence of iron/aluminum oxides and aggregates on plant available water with different amendments in red soils. J Soil Water Conserv 74:145–159. https://doi:10.2489/jswc.74.2.145

55. Repsiene R, Karcauskiene D (2016) Changes in the chemical properties of acid soil and aggregate stability in the whole profile under long-term management history. Acta Agric Scand Sect B 66:671–676. https://doi:10.1080/09064710.2016.1200130

56. Douch J, Hamdani M, Fessi H, Elaissari A (2009) Acid-base behavior of a colloidal clays fraction extracted from natural quartz sand: effect of permanent surface charge. Colloids Surf, A. 338:51–60. https://doi:10.1016/j.colsurfa.2008.12.034

57. Barral M, Arias M, Guerif J (1998) Effects of iron and organic matter on the porosity and structural stability of soil aggregates. Soil Tillage Res 46:261–272. https://doi:10.1016/s0167-1987(98)00092-0

58. Walker TW, Adams AFR (1958) Studies on soil organic matter: I. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. Soil Sci 85:307–318.
59. Cui Y, Xiao R, Xie Y, Zhang M (2018) Phosphorus fraction and phosphate sorption-release characteristics of the wetland sediments in the Yellow River Delta. Phys Chem Earth, Parts A/B/C 103:19-27. https://doi:10.1016/j.pce.2017.06.005

60. Gottselig N, Amelung W, Kirchner JW, Bol R, Eugster W, Granger SJ, Hernández-Crespo C, Herrmann F, Keizer JJ, Korkiakoski M (2017) Elemental composition of natural nanoparticles and fine colloids in European forest stream waters and their role as phosphorus carriers. Global Biogeochem Cycles 31:1592–1607. https://doi:10.1002/2017GB005657

61. Liu J, Yang JJ, Liang XQ, Zhao Y, Cade-Menun BJ, Hu YF (2014) Molecular speciation of phosphorus present in readily dispersible colloids from agricultural soils. Soil Sci Soc Am J 78:47–53. https://doi:10.2136/sssaj2013.05.0159

62. Petter FA, Madari BE, da Silva MAS, Carneiro MAC, de Melo Carvalho MT, Júnior BHM, Pacheco LP (2012) Soil fertility and upland rice yield after biochar application in the Cerrado. Pesqui Agropecu Bras 47:699–706. https://doi:10.1590/S0100-204X2012000500010

63. van der Grift B, Osté L, Schot P, Kratz A, van Popta E, Wassen M, Griffioen J (2018) Forms of phosphorus in suspended particulate
matter in agriculture-dominated lowland catchments: Iron as phosphorus carrier. Sci Total Environ 631:115-129.

https://doi:10.1016/j.scitotenv.2018.02.266

64. Gottselig N, Nischwitz V, Meyn T, Amelung W, Bol R, Halle C, Vereecken H, Siemens J, Klumpp E (2017) Phosphorus binding to nanoparticles and colloids in forest stream waters. Vadose Zone J 16.

https://doi:10.2136/vzj2016.07.0064

65. Missong A, Bol R, Nischwitz V, Krüger J, Lang F, Siemens J, Klumpp E (2018) Phosphorus in water dispersible-colloids of forest soil profiles. Plant soil 427:71–86. https://doi:10.1007/s11104-017-3430-7

66. Pokrovsky OS, Dupré B, Schott J (2005) Fe–Al–organic colloids control of trace elements in peat soil solutions: results of ultrafiltration and dialysis. Aquat Geochem 11:241–278.

https://doi:10.1007/s10498-004-4765-2

67. Leon A, Kohyama K (2017) Estimating nitrogen and phosphorus losses from lowland paddy rice fields during cropping seasons and its application for life cycle assessment. J Cleaner Prod 164:963-979.

https://doi:10.1016/j.jclepro.2017.06.116
Table 1: Basic chemical and physical properties of soil, and characterization of sites located in Zhejiang Province, China.

| Soils | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10 | S11 | S12 | S13 | S14 | S15 |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| pH    | 3.9 | 4.5 | 5.1 | 5.2 | 5.3 | 5.5 | 5.9 | 6.2 | 6.4 | 6.6 | 7.0 | 7.1 | 7.3 | 7.4 | 7.8 |
| TC (g kg\(^{-1}\)) | 8.4 | 3.3 | 13.1 | 11.1 | 14.8 | 4.5 | 10.3 | 7.7 | 17.5 | 13.5 | 5.2 | 20.2 | 9.0 | 14.1 |
| TN (g kg\(^{-1}\)) | 1.5 | 0.5 | 1.4 | 1.7 | 0.6 | 1.4 | 1.3 | 0.9 | 2.0 | 1.4 | 0.7 | 2.2 | 0.9 | 1.7 |
| TP (g kg\(^{-1}\)) | 1.3 | 0.2 | 0.5 | 0.8 | 1.1 | 0.3 | 0.8 | 0.7 | 1.6 | 0.6 | 0.5 | 0.3 | 1.1 | 0.9 | 1.0 |
| CEC (cmol kg\(^{-1}\)) | 118.2 | 123.1 | 94.7 | 127.1 | 104.2 | 110.0 | 116.7 | 71.4 | 91.6 | 116.8 | 126.4 | 164.8 | 23.5 | 31.7 | 29.7 |
| Sand (%) | 12.4 | 45.5 | 71.8 | 56.9 | 16.4 | 70.6 | 18.9 | 69.4 | 78.5 | 32.4 | 21.4 | 70.7 | 46.0 | 16.6 | 9.8 |
| Silt (%) | 82.1 | 18.1 | 14.9 | 28.8 | 42.8 | 7.5 | 42.3 | 18.4 | 10.7 | 44.8 | 40.6 | 13.6 | 25.7 | 44.7 | 82.6 |
| Clay (%) | 5.5 | 36.4 | 13.3 | 14.3 | 40.9 | 21.9 | 38.8 | 12.2 | 10.8 | 22.8 | 38.0 | 15.8 | 28.3 | 38.7 | 7.7 |
| Land use and vegetation | Orchard | Orchard | Single cropping | Vegetable | Double cropping | Rice-rap | Single cropping | Rice-rap | Single cropping | Rice-wheat | Rice-rap rotation | Rice-wheat rotation |
| Mean annual temperature (°C) | 16.4 | 17.0 | 16.5 | 16.3 | 18.0 | 17.7 | 17.0 | 17.8 | 16.5 | 16.0 | 15.8 | 16.8 | 16.4 | 14.0 | 16.9 |
| Annual precipitation (mm) | 1814.0 | 1657.0 | 895.6 | 1373.6 | 1670.1 | 1760.1 | 1489.0 | 1474.1 | 1552.0 | 1412.7 | 1200.0 | 1632.9 | 1446.8 | 1700.0 | 1486.0 |
| Geographic coordinates (N, E) | 118°16', 118°50' | 119°4', 29°5' | 120°23', 27°30' | 118°26', 28°54' | 119°8', 29°44' | 122°41', 29°48' | 122°17', 30°2' | 120°11', 29°9' | 120°47', 27°16' | 28°19' |
Table 2
The total dissolved P (TDP), and truly soluble P (TSP), colloidal P, colloidal P/TDP, and ratio of TDP to soil total P (TP) in the supernatant of different soils after wet sieving.

| Soil | TDP (mg kg⁻¹) | TSP (mg kg⁻¹) | Colloidal P (mg kg⁻¹) | Colloidal P/TDP (%) | TDP/TP (%) |
|------|---------------|---------------|-----------------------|---------------------|------------|
| S1   | 5.71±0.12     | 2.62±0.34     | 3.1±0.34              | 54.17               | 0.44       |
| S2   | 3.49±0.22     | 0.79±0.18     | 2.7±0.22              | 77.27               | 1.75       |
| S3   | 6.35±2.02     | 1.59±0.45     | 4.76±1.57             | 75.00               | 1.27       |
| S4   | 3.41±0.11     | 1.83±0.11     | 1.59±0.22             | 46.51               | 0.43       |
| S5   | 6.03±0.45     | 2.78±0.34     | 3.25±0.11             | 53.95               | 0.55       |
| S6   | 2.14±0.56     | 1.43±0.67     | 0.71±0.11             | 33.33               | 0.71       |
| S7   | 1.27±0.22     | 0.87±0.11     | 0.4±0.34              | 31.25               | 0.16       |
| S8   | 5.48±0.11     | 0.87±0.11     | 4.6±0.24              | 84.06               | 0.78       |
| S9   | 29.92±0.34    | 27.38±0.11    | 2.54±0.22             | 8.49                | 1.87       |
| S1   | 3.73±0.56     | 0.79±0.15     | 2.94±0.56             | 78.72               | 0.62       |
| S1   | 5.4±0.45      | 2.18±0.62     | 3.21±1.07             | 59.56               | 1.08       |
| S1   | 1.90±0.45     | 1.35±0.56     | 0.56±0.11             | 29.17               | 0.63       |
| S1   | 7.06±0.11     | 4.05±0.11     | 3.02±0.22             | 42.70               | 0.64       |
| S1   | 6.59±0.14     | 4.13±0.90     | 2.46±1.01             | 37.35               | 0.73       |
| S1   | 6.43±0.11     | 2.14±1.01     | 4.29±0.90             | 66.67               | 0.64       |

Data represent the average of three replicates ± standard deviations.
Table 3
The colloidal P content (mg kg\(^{-1}\)) of different-sized soil aggregates and the contribution rate of aggregates to colloidal P (CR) in the total 15 soils.

| Size of aggregates | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10 | S11 | S12 | S13 | S14 | S15 |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Colloidal 2–8 mm   | 14.04 | 1.25 | 2.05 | 11.35 | 6.10 | 11.01 | 1.78 | 16.35 |
| P 0.26–2 mm        | 15.55 | 1.55 | 7.22 | 11.33 | 8.98 | 17.11 | 23.63a | 1.27b | 3.85 | 12.50 |
| 0.053–0.26 mm      | 6.88b | 9.07b | 4.48c | 2.14c | 2.25a | 2.2b | 2.87b | 5.31c | 12.70a |
| <0.053 mm          | 1.67c | 5.55c | 5.60b | 3.45a | 5.55c | 12.3c | 1.00c | 4.50a | 7.69b |
| CR           | 2–8 mm | 4.46c | 2.17 | 1.52 | 4.10c | 0.83c | 4.43d | 5.37 | 4.78d | 3.86d | 3.18d | 4.39 | 2.58d | 3.96c | 6.25d | 11.33 |
|       | c     | d     | b     | b     | a     |
|-------|-------|-------|-------|-------|-------|
| 0.26–2 mm | 10.75 | 11.7  | 7.03  | 11.02 | 10.2  |
|        |       |       |       |       | 12.30 |
|        | 6.18  |       |       |       |       |
| b     | 2a    | c     | b     | 5.05b | 5.88c |
|       | 5.39c | a     | 4.51c | a     | 5.64b |
|       | 4.51b | 7.61c | 5.51c |
| 0.053–0.26 mm | 12.8  | 10.2  | 10.17 | 14.37 | 9.66  |
|        |       |       |       |       | 2.04  |
| 0.053–0.26 mm | 9.47b | 6a    | 7b    | b     | a     |
|        | 7.98b | a     | 7.41c | 10.38a| 5.82b |
|        | 4.17c | 9.34a | 9.81b | 2.69d |
| <0.053 mm | 26.14 | 11.2  | 15.9  | 20.79 | 15.06 |
|        | 19.87 | 4.43  |       |       |       |
|        | 4.07  |       |       |       |       |
| <0.053 mm | a     | 8a    | 2a    | a     | a     |
|        | a     | a     | a     | b     | 9.11b |
|        | 9.21a | 8.04a | b     | 8.80a | 8.32a |
|        | 10.37a| 7.11b |

Letters in different sized aggregates indicated significant difference at $P < 0.05$ level.
Table 4
Results from correlation analyses and stepwise linear regressions of colloidal P and colloidal P/TDP in different sized aggregate with soil aggregate associated mean weight diameter (MWD), geometric mean diameter (GMD), pH, total carbon (TC), total nitrogen (TN), C/P, C/N, Al, Fe, and Ca. * P < 0.05. ** P < 0.01. Total=total soil aggregate sizes.

| Indexes   | Aggregate sizes | TC  | TN  | TP     | pH    | Al    | Fe    | Ca     | C/P    | C/N    | MWD   | GMD   |
|-----------|-----------------|-----|-----|--------|-------|-------|-------|--------|--------|--------|-------|-------|
| **Colloidal P** |                 |     |     |        |       |       |       |        |        |        |       |       |
| Total     |                 | 0.151 | 0.134 | 0.706* | 0.223 | 0.220* | *     | 0.251* | 0.269* | -0.424* | 0.241 | 0.224* | -0.101 |
| 2–8 mm    |                 | 0.302 | 0.328 | 0.769* | 0.095 | 0.374* | 0.464* | *       | *       | 0.492* | -0.517* | 0.118 | 0.136 | -0.247 |
| 0.26–2mm  |                 | 0.089 | 0.129 | 0.012  | 0.277 | 0.317 | 0.142 | *       | *       | *       | 0.102 | 0.102 | -0.264 |
| 0.053–0.26mm |             | 0.149 | 0.128 | 0.631  | 0.311 | 0.204 | 0.190 | 0.085  | 0.461* | -0.295 | 0.290 | -0.124 |
| <0.053mm  |                 | 0.282 | 0.101 | 0.344  | **    | -0.115 | -0.085 | *       | *       | 0.541  | 0.528* | 0.189 |
| **Colloidal P/TDP** |               |     |     |        |       |       |       |        |        |        |       |       |
| Total     |                 | *   | *   | 0.026  | *     | 0.474* | *     | *       | *       | *       | -0.072 | -0.056 |
| 2–8 mm    |                 | -0.509 | -0.452 | 0.315  | -0.387 | 0.764** | *     | -0.441 | *       | -0.435 | -0.048 | -0.030 |
| 0.26–2mm  |                 | -0.645* | -0.547* | 0.147  | -0.436 | 0.741** | 0.513  | *       | -0.632* | -0.411 | -0.090 | -0.097 |
| 0.053–0.26mm |             | *   | -0.547* | 0.147  | -0.436 | 0.741** | 0.513  | *       | -0.632* | -0.411 | -0.090 | -0.097 |
| <0.053mm  |                 | -0.485 | -0.429 | 0.008  | -0.435 | 0.515* | 0.458  | *       | -0.364 | -0.234 | -0.082 | -0.025 |
|           |                 | -0.386 | -0.455 | 0.322  | 0.107  | 0.247  | 0.104  | 0.261  | -0.013 | 0.002  | -0.074 | -0.086 |
| Stepwise linear regression | Colloidal P |  |  |  |  | Colloidal P/TDP |  |  |  |
|---------------------------|------------|---|---|---|---|----------------|---|---|---|
| $R^2$                     | 0.605      | 44.116 | <0.001 | 0.539 | 16.058 | <0.001 |
| $B$                       | -8.017     | -2.979 | 0.004  | 0.478 | 2.98 | 0.004  |
| $t$                       | 0.087      | 3.235  | 0.018  | -0.028 | -6.947 | <0.001 |
| $P$                       | 10.598     | 3.88   | <0.001 | 0.015 | 4.883 | <0.001 |
| Al                        | -2.257     | 3.634  | 0.004  | 0.413 | 3.911 | <0.001 |
| TN                        | 5.977      | -2.651 | <0.001 | -0.363 | -2.057 | 0.044  |
| MWD                       | 5.977      | -2.651 | <0.001 | -0.363 | -2.057 | 0.044  |
Figure 1 Location of sampling sites. Location sites of S1-S15 correspond to Kaihua, Kecheng, Longquan, Zhuji, Lingxi, Changshan, Qiandaohu, Liandu, Tonglu, Zhoushan, Wuxing, Tiantai, Shengzhou, Mazhan, and Luqiao in Zhejiang province, China, respectively.
Figure 2 Soil total P fraction (a), colloidal P (P_{coll}) fraction (b) and loss potential (c) of three sized aggregates (2–8 mm, 0.26–2 mm, 0.053–0.26 mm) and (silt+clay) particles (<0.053 mm) in 15 soils.
Figure 3 Linear relationship between colloidal P (P_{coll}) content and soil parameters (b) of three sized aggregates (2–8 mm, 0.26–2 mm, 0.053–0.26 mm) and (silt+clay) particles (<0.053 mm).
Figure 4 Soil colloidal phosphorus content (a) and loss potential (b) of three sized aggregates (2–8 mm, 0.26–2 mm, 0.053–0.26 mm) and (silt+clay) particles (<0.053 mm) under different land use types. OR = orchard soils (n=3); RICE = Rice fields including single and double cropping soils (n=6); RDR = Rice and dry land rotation including rice-rape rotation and rice-wheat rotation soils (n=3); VE = vegetable soils (n=3); Capital letters indicated significant difference among different sizes of aggregate at $P < 0.05$ level.
Lowercase indicated significant difference of the same size of aggregate among land use types at $P < 0.05$ level.
Supporting Information:

Table S1
The total phosphorus (TP, g kg\(^{-1}\)), dissolved P (TDP, mg kg\(^{-1}\)), and truly soluble P (TSP, mg kg\(^{-1}\)) of different-sized soil aggregates in the total 15 soils.

| Size of aggregates | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10 | S11 | S12 | S13 | S14 | S15 |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 2–8 mm             | 1.48 | 0.27 | 0.52 | 0.86 | 1.00 | 0.26 | 0.93 | 0.59 | 1.24 | 0.64 | 0.54 | 0.29 | 1.00 | 0.88 | 0.8 |
| 0.26–2 mm          | 1.58 | 0.20 | 0.66 | 0.93 | 1.48 | 0.27 | 0.87 | 0.89 | 2.45 | 0.7 | 0.57 | 0.28 | 1.24 | 0.98 | 1.16 |
| 0.053–0.26 mm      | 1.32 | 0.16 | 0.38 | 0.59 | 0.97 | 0.29 | 0.76 | 0.78 | 1.25 | 0.63 | 0.60 | 0.28 | 0.98 | 1.03 | 1.87 |
| <0.053 mm          | 0.94 | 0.18 | 0.53 | 0.43 | 0.67 | 0.21 | 0.77 | 0.7 | 0.95 | 0.58 | 0.31 | 0.31 | 0.59 | 0.79 | 0.57 |
| 2–8 mm             | 24.87 | 1.85 | 4.45d | 18.05 | 4.41c | 4.6 | 9.93c | 18.3b | 38.57 | 3.89b | 3.91 | 3.09 | 13.71 | 16.36a | 25.46a |

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| Size          | TS | P   |
|--------------|----|-----|
| 0.053–0.26 mm| 12.62 | 2.29 |
|              | 9.33b | 12.57 |
|              | 7.64b | 2.83 |
|              | 11.21 | 19.42 |
|              | 29.30 | 5.49a |
|              | 2.29  | 3.76 |
|              | 18.64 | 15.63a |
|              | 14.45c |
| <0.053 mm    | 2.21c | 1.42c |
|              | 6.52c | 6.25c |
|              | 7.37b | 4.05 |
|              | ab    | 6.38d |
|              | 8.24c | 18.57 |
|              | c     | 3.29c |
|              | 9.40a | 5.38c |
|              | a     | 9.81c |
|              | 12.76b|
|              | 22.54a|
| TS           | 0.83b | 0.59c |
| 2–8 mm       | 2.41c | 6.69a |
|              | 2.14c | 0.59 |
|              | ab    | 2.51b |
|              | 7.3c  | 8.71b |
|              | c     | 2.41b |
|              | 2.13c | 2.10c |
|              | 3.71c | 2.11a |
|              | d     | 4.10c |
| P            | 1.56a | 0.79c |
| 0.26–2 mm    | 8.43a | 6.59a |
|              | 3.15b | 0.56 |
|              | ab    | 2.38b |
|              | 7.46b | 10.29 |
|              | c     | 2.67b |
|              | 2.19c | 1.91c |
|              | 8.31a | 1.97ab|
|              | b     | 5.24b |
| 0.053–0.26 mm| 0.75c | 0.51c |
|              | 7.23b | 3.49b |
|              | 3.16b | 0.69 |
|              | ab    | 2.84a |
|              | 8.21a | 6.62c |
|              | c     | 3.01a |
|              | 2.71c | 2.65 |
|              | 4.14b | 1.83b |
|              | a     | 4.28c |
| <0.053 mm    | 0.53d | 0.56 |
|              | 1.25d | 0.70c |
|              | 4.77a | 1.46 |
|              | ab    | 2.79a |
|              | 7.68b | 6.26c |
|              | 2.22c | 1.80 |
|              | 0.88c | 2.12d |
|              | c     | 2.54a |
|              | c     | 7.31a |

Letters in different sized aggregates indicated significant difference at $P<0.05$ level.
## Table S2
Correlation analysis of colloidal P in TP in soil aggregates and soil aggregate associated MWD, GMD, pH, TC, TN, C/N, C/P, Al, Fe, and Ca (n=45). MWD=mean weight diameter, MWD=mean weight diameter, TC=total C, TN=total N.

| Indexes | Ratio of colloidal P to TP | 2–8 mm | 0.26–2 mm | 0.053–0.26 mm | <0.053 mm | MWD | GMD | pH | TC | TN | TP | C/N | C/P | Al | Fe | Ca |
|---------|---------------------------|--------|-----------|--------------|-----------|-----|-----|----|----|----|----|-----|-----|----|----|----|
| Ratio of colloidal 0.26–2 mm to TP | | | | | | | | | | | | | | | | | |
| 0.577** | 1 | | | | | | | | | | | | | | | | |
| 0.614** 0.662** | 1 | | | | | | | | | | | | | | | | |
| 0.053–0.26 mm | | | | | | | | | | | | | | | | | |
| 0.152 | -0.115 | -0.246 | 1 | | | | | | | | | | | | | | |
| 0.241 | -0.035 | -0.055 | **0.584** | 1 | | | | | | | | | | | | | | |
| 0.001 | -0.236 | -0.224 | **0.401** | 0.461 | 1 | | | | | | | | | | | | | | |
| 0.207 | -0.033 | -0.133 | **0.710** | 0.314 | 0.411 | 1 | | | | | | | | | | | | | |
| -0.245 | **-0.378** | 0.291 | 0.236 | **0.452** | 0.002 | 0.419 | 1 | | | | | | | | | | | | | |

| MWD | GMD | pH | TC | TN | TP | C/N | C/P | Al | Fe | C |
|-----|-----|----|----|----|----|-----|-----|----|----|----|
|     |     |    |    |    |    |     |     |    |    |    |
| 0.359 | -0.018 | -0.054 | 0.092 | 0.196 | -0.135 | -0.076 | 0.162 | **0.330** | 1 | | | | | | | | |
| -0.337 | -0.340 | -0.219 | -0.047 | **-0.29** | **0.540** | **0.729** | **0.586** | 0.243 | -0.108 | 1 | | | | | | | | |
| 0.247 | -0.270 | -0.020 | -0.346 | 0.295 | 0.148 | 0.240 | **0.478** | **0.385** | **-0.503** | **0.438** | 1 | | | | | | | |

* P < 0.05, ** P < 0.01
|    | Al       | Fe       | Ca       |
|----|----------|----------|----------|
|    | -0.186   | -0.318   | 0.318    |
|    | 0.130    | -0.345   | -0.042   |
|    | 0.065    | 0.147    | -0.162   |
|    | -0.536** | -0.310   | 0.532**  |
|    | -0.038   | -0.235   | 0.152    |
|    | 0.077    | -0.112   | 0.162    |
|    | -0.536** | -0.222   | 0.429    |
|    | -0.280   | 0.014    | 0.443    |
|    | *        | 0.098    | 0.413    |
|    | *        | 0.443    | *        |
|    | 7*       | *        | **       |
|    | -0.32    | -0.30    | -0.446   |
|    | -0.189   | -0.189   | -0.22    |
|    | -0.32    | -0.30    | -0.22    |
|    | 1        | 1        | 2        |

* Significant correlation at the 0.05 level (two-tailed). ** Significant correlation at the 0.01 level (two-tailed)
**Figure S1** Water-stable aggregate (WSA) and Dry soil aggregates (DSA) fractions of soils.
Figure S2 Mean weight diameter (MWD) and geometric mean diameter (WMD) of different soil aggregates. Letters indicated significant difference among soils at $P < 0.05$ level.