Organic Carbon Linkage with Soil Colloidal Phosphorus at Regional and Field Scales: Insights from Size Fractionation of Fine Particles

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Organic carbon linkage with soil colloidal phosphorus at regional and field scales: insights from size fractionation of fine particles

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ABSTRACT: Nano and colloidal particles (1–1000 nm) play important roles in phosphorus (P) migration and loss from agricultural soils; however, little is known about their relative distribution in arable crop soils under varying agricultural geo-landscapes at the regional scale. Surface soils (0–20 cm depth) were collected from 15 agricultural fields, including two sites with different carbon input strategies, in Zhejiang Province, China, and water-dispersible nano colloids (0.6–25 nm), fine colloids (25–160 nm), and medium colloids (160–500 nm) were separated and analyzed using Asymmetrical Flow Field Flow Fractionation (AF4) technique. Three levels of fine-colloidal P content (3583–6142, 859–2612, and 514–653 μg kg^{-1}) were identified at the regional scale. The nano colloidal fraction correlated with organic carbon (C_{org}) and calcium (Ca), and the fine colloidal fraction with C_{org}, silicon (Si), aluminum (Al), and iron (Fe). Significant linear relationships existed between colloidal P and C_{org}, Si, Al, Fe, Ca, and for nano-colloidal P with Ca. The organic carbon controlled colloidal P saturation, which in turn affected the P carrier ability of colloids. Field-scale organic carbon inputs did not change the overall morphological trends in size fractions of water dispersible colloids. However, they significantly affected the peak concentration in each of the nano-, fine- and medium-colloidal P fractions. Application of chemical fertilizer with carbon-based solid manure and/or modified biochar reduced the soil nano-, fine- and medium-colloidal P content by 30–40%; however, application of chemical fertilizer with biogas slurry boosted colloidal P formation. This study provides a deep and novel understanding of the forms and composition of colloidal P in agricultural soils and highlights their spatial regulation by soil characteristics and carbon inputs.

Keywords: organic carbon; nano-colloidal phosphorus; colloidal phosphorus; Asymmetric Flow Field-Flow Fractionation; biochar; regional scale; field scale

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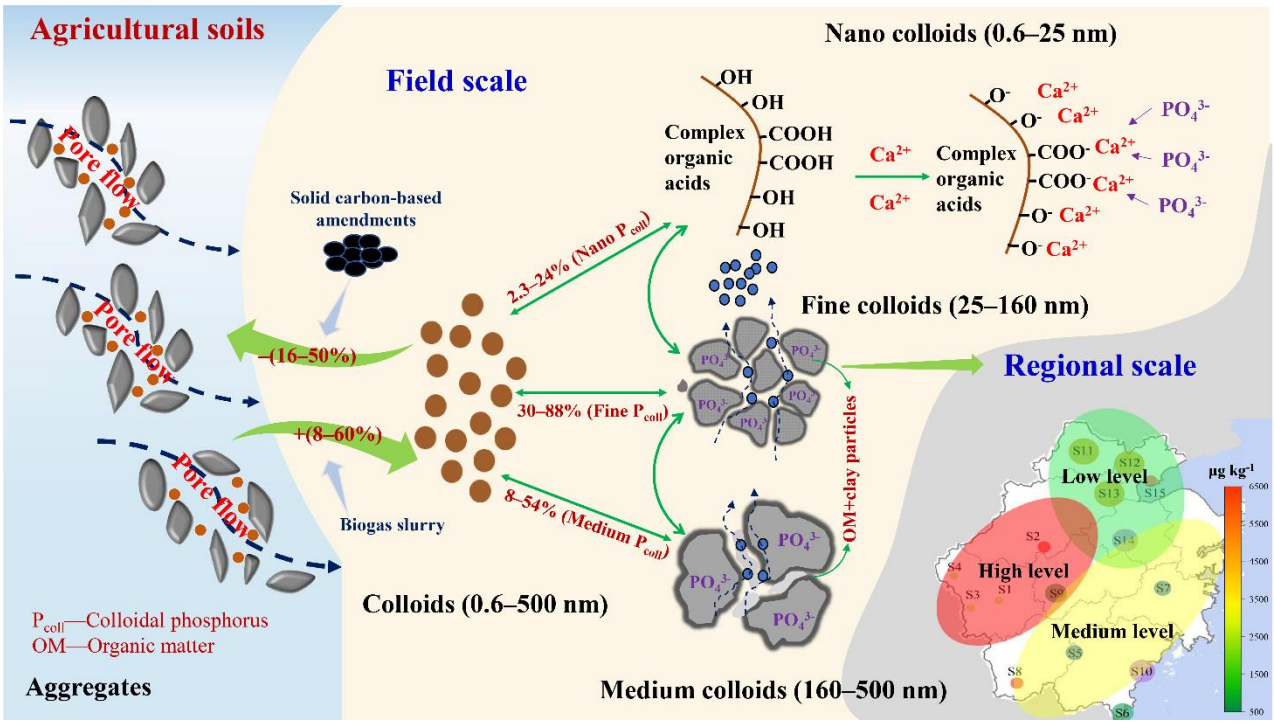

■ INTRODUCTION

Loss of soil phosphorus (P) from agricultural fields has long been a global challenge, and is one of the main factors responsible for the eutrophication of rivers and lakes in southern China[1,2]. However, the formation, distribution, and migration mechanisms of P in agricultural soils remain inadequately understood, which presents great difficulties in source control mitigation where P is present as a diffuse and non-point pollution source[3]. Previous studies suggest that the majority of P in soil exists as larger-sized particulate and conventionally dissolved P, which are usually distinguished using a 0.45 μm filter membrane[4,5] However, this consideration neglects the important role of colloidal P (~1–1000 nm) and its subfraction nano-colloidal P (~1–100 nm) in the process of soil P transport. In fact, colloidal P forms have special colloid-chemical properties[6,7], which are fundamentally different from that of truly dissolved P. In general, colloidal size fractions like nano colloids (~1–100 nm), fine colloids (~100–200 nm), and medium colloids (~200–500 nm) have a large specific surface area and are rich in charge density, and, thus, they can immobilize P and other compounds[8,9]. The colloidal P can potentially migrates faster than truly dissolved P due to the spatial exclusion and electrostatic repulsion of the soil matrix[10]. Moreover, some studies have found that the P molecules carried by colloids with low bioavailability and small particle size enhance migration ability and contribute to the transport of soil P to external water bodies[11–13]. Overall, the migration and transformation of colloidal P in agricultural soil systems is of crucial environmental and ecological significance.

Related studies in acid forest river systems have indicated that organic carbon (C_{org}), iron (Fe), and aluminum (Al) may be the main binding elements of nano-, fine- and medium-colloidal P, and

that the elemental composition may vary with the specific location within or between rivers[14–15]. However, detailed colloidal P fluxes remain almost blind spots in some areas of ecosystem research[16]. In agricultural systems, colloidal carriers may be affected by farm management practices, such as irrigation, fertilization, and land-use types[12,17]. Compared to forest soils, agricultural soils usually have lower organic matter, higher inorganic mineral contents, and relatively stable seasonal input and outputs[18]. Therefore, the composition and morphology of the colloids in agricultural soils may be very different from those in natural ecosystems, e.g., forest and steppe soils. The majority of previous research on colloidal P in agricultural systems has focused on the whole colloidal size range (1–1000 nm) and single, specific farm types[17], and only few studies have separated nano-, fine- and medium-colloidal P[19,20]. The rarest studies are those that also provide specific nutrient compositions of colloidal particle size ranges[21,22]. The emergence of the Asymmetrical Flow Field Flow Fractionation (AF4) technique enables us to separate and study nano-, fine- and medium-colloidal P[23]. As an important advantage of this technique, crude extracts of soil water dispersible colloids can be analyzed with the only requirement that the upper particle size is limited to about 500 nm by centrifugation. The composition of nano-, fine- and medium-colloidal P in stream waters and soils has been explored using this technique[24,20,25,26].

Input of carbon-based fertilizers directly affects the forms, composition, and loss of P in agricultural soils[27,28]. Organic fertilizer (manures) usually contains a large amount of organic P, which may increase the soil P level after being applied to the soil[29]. However, it may also reduce the release of soluble P and improve soil bacteria activity compared with chemical fertilizers[2,30]. Carbon-based biochar has recently been recommended as an amendment that effectively improves

soil physical and chemical conditions and increases crop productivity[31–32]. However, biochar contains a large amount of soluble P, which may increase the loss of soil P once applied to the soil[33]. Conversely, it is rich in pore structures, specific surface area, and functional groups, which may have the potential to immobilize soil P[33,34]. However, regardless of the type of fertilization, organic carbon input will participate in the formation of soil colloidal particles after entering the soil, whereby some may be adsorbed by the colloids, and some may have colloidal properties (i.e., high molecular mass organic acids). At present, there are very few reports on the elemental compositions and morphologies of soil colloidal P under different carbon input strategies.

Therefore, we collected soil samples covering typical agricultural ecosystems from 15 sites throughout Zhejiang Province, China, to explore the influence of organic carbon and other soil parameters on nano-, fine- and medium-colloidal P at the regional scale. At the field scale, two experimental stations were established to evaluate the effects of different carbon input strategies on the soil P forms, content, and loss potential. Soil samples were analyzed using AF4 coupled to inductively coupled plasma-mass spectrometry (ICP–MS) and to an online organic carbon detector (OCD). We hypothesized that, due to different formation mechanisms: i) At the regional scale, specific distribution patterns of nano-, fine- and medium-colloidal P occur under different land-use types, and organic carbon affects the ability of colloids and nanoparticles to bind P; ii) at the field scale, chemical fertilizer will increase soil nano-, fine- and medium-colloidal P concentrations; however, carbon inputs (including organic fertilizer and biogas slurry, with or without modified biochar) will reduce the soil nano-colloidal and fine/medium-colloidal P contents.

■ MATERIALS AND METHODS

108 **Site description and soil sampling.** Soil samples were taken from 15 representative sites (H1–
109 H5, M1–M5, and L1–L5) with different land-use types in Zhejiang Province (Figure S1), China,
110 which included 6 agricultural planting types with conventional fertilization regimes (Tables S1 and
111 S2), which were almost evenly distributed in the county-level administrative area of the region
112 (1,055,00 km^2, Figure S1). In each sampling site, three representative fields (three replicates) of the
113 same crop type were selected, and 10-kg topsoil samples (0–20 cm depth) were randomly collected
114 from 10–15 points of each field without regard to soil horizons using S-shaped sampling method[35]
115 and then mixed to prepare a representative sample of the whole field. Samples were air-dried and
116 divided into two portions: One portion was ground through a 0.154-mm sieve for determining the
117 basic physical and chemical properties, and the other portion was carefully broken into small pieces
118 by hand and passed through an 8-mm sieve for separation of water-dispersible colloids (WDC) and
119 determination of colloidal fractions. All the soil samples were stored in sealed plastic bags and kept
120 at 4°C before analysis. Detailed descriptions of sites and soils are shown in Table S1.

121 In addition, two experimental crop rotation field stations were established as part of the study
122 (Figure S1), i.e., in Site 1 (double-season rice) and Site 2 (rice-wheat rotation). Three treatments
123 with the same P application rate and a control (CK) were applied at each site. The P application rates
124 were 68.8 kg P ha^{-1} per year (two seasons) at Site 1 and 103.2 kg P ha^{-1} per year (two seasons) at
125 Site 2. The treatments in Site 1 were: a) Control without fertilization (CK), b) Conventional
126 fertilization (chemical fertilizer, CF), c) Chemical fertilizer + organic fertilizer (sheep manure) (OF),
127 and d) Chemical fertilizer + organic fertilizer + modified rice straw biochar (OFSB). The treatments
128 in Site 2 were: e) Control without fertilization (CK), f) conventional fertilization (CF), g) Chemical

129 fertilizer + biogas slurry (BS), and h) Chemical fertilizer + biogas slurry + modified rice straw
130 biochar (BSSB). Each treatment was repeated three times with a total of 12 completely random plots
131 (33.3 m^2 per each). Physicochemical properties of modified biochar, organic fertilizer, and biogas
132 slurry are shown in Table S3. The specific nutrient contents of fertilizers and the fertilization
133 schedule are shown in Tables S4 and S5, respectively. Five soil samples from the 0–20 cm soil layer
134 in each plot were randomly collected after harvesting in the second season and were mixed into a 5-
135 kg sample, transported to the laboratory, air-dried, and again divided into two equal portions (i.e., as
136 for all main site samples). One portion was ground through a 0.154-mm sieve to determine basic
137 physical and chemical properties. The other portion was carefully broken into small pieces by hand
138 and passed through an 8-mm sieve for separation of WDC.

139 **Soil physical and chemical parameters.** Soil particle size distribution was determined using
140 the hydraulic method according to the international soil texture classification[36]. Soil pH was
141 determined using a glass electrode pH meter (PHS-3C, Shanghai) at a solid-to-liquid ratio of 1:5.
142 Soil cation exchange capacity (CEC) was determined using ammonium acetate (12.5 mL 1 M
143 NH_4OAc, 2.5 g soil)[37]. Samples were digested with H_2SO_4-$HClO_4$, and the total P contents were
144 determined using molybdenum blue colorimetric method[38]. Soil total carbon (TC) and total nitrogen
145 (TN) were measured using an element analyzer (Elementar, Vario MAX CNS, Germany). The
146 bioavailable fraction of Fe, Al, and Ca was determined using an Inductively Coupled Plasma-Mass
147 Spectrometer (ICP-MS; Agilent 7500, Agilent Technologies, Japan) after extraction by 0.2 mol L^{-1}
148 oxalic acid (2.5 g soil, 50 mL oxalic acid)[39]. Soil available P (Olsen P) was extracted with 0.5 M
149 $NaHCO_3$ (pH 8.5)[40] and analyzed using molybdenum blue colorimetry.

150 **Water dispersible colloids extraction.** The water-dispersible soil colloids were extracted by
151 the method of Sequaris and Lewandowski [41], which avoids the destruction of small aggregates in
152 soil[25], and mainly includes two procedures of shaking and sedimentation at a water to soil mass ratio
153 of 1 to 8 to obtain WDC suspension. All samples were extracted and analyzed in triplicate. In brief,
154 10 g soil subsamples (d < 8 mm) were mixed with 20 mL deionized water and shaken on a horizontal
155 shaker at 150 rpm for 6.5 h. The suspension was then diluted four times with deionized water and
156 left to stand to allow sedimentation (approximately 6 min, according to Stokes' law, particle density
157 was assumed as 1.5 g cm^{-3}) to remove the particles > 20 μm[10,42]. Then, the supernatant was
158 transferred into a 25-mL centrifuge tube, and the suspension (< 20 μm) was centrifuged at 4000 rpm
159 for approximately 7 min. The centrifugation time was calculated according to Hathaway (1956) to
160 obtain WDC with a particle size of < 500 nm in the supernatant[43,44]. Irrigation experiments with
161 undisturbed mesocosm soil columns demonstrated that the leached colloids had a maximum size of
162 400 nm[26]. For additional details see supplementary information (section S1). In addition, dynamic
163 light scattering (DLS) measurements (Malvern Nano-ZS, Malvern Panalytical, UK) were performed
164 on the separated WDC samples to verify the effectiveness of the size fractionation, and the z-average
165 of diameter was calculated based on the intensity distribution (Table S6)[43,19].

166 The truly dissolved elemental fractions were determined by ultrafiltration using a molecular
167 weight cut off of 3 kDa (Millipore, USA). 15 mL of WDC suspension were centrifuged at 4000 g
168 for 15 min. The permeate was collected and analyzed by ICP-MS and OCD for P, Fe, Al, Si, Ca, Mg
169 and organic carbon.

170 **Asymmetric Flow Field Flow Fractionation**

171 Size resolved elemental characterization of the colloidal fractions in the extracted WDCs was
172 achieved by AF4 (AF2000, Postnova Analytics, Landsberg, Germany) using a 1 kDa PES membrane,
173 1 mL injection volume, 20 min focusing time and a separation time of 91 min including a cross flow
174 gradient from 2.5 mL min^{-1} down to zero. The AF4 was coupled online to a UV-vis detector
175 (Postnova Analytics), a DLS detector (Malvern Nano-ZS, Malvern Panalytical, UK) and a C_{org}
176 detector (OCD; DOC laboratory Dr. Huber, Germany) for quantification of the particulate organic
177 carbon using multipoint external calibration and determination of the particle size. In parallel, the
178 same AF4 method was run with ICP-MS detection (Agilent 7500, Agilent Technologies, Japan) for
179 quantification of the particulate elemental concentrations monitoring ^{31}P, ^{56}Fe, ^{27}Al, ^{28}Si, ^{44}Ca and
180 ^{24}Mg using He collision cell mode. A post channel calibration approach was applied using Rh as the
181 internal standard which was developed and validated in our previous work[45,46]. Latex particles were
182 analyzed with the same AF4 method as size markers for the obtained colloidal fractions (Figure S2).
183 Further experimental details are given in the supporting information (section S2, Table S7 and Table
184 S8).

185 **Colloidal Phosphorus Saturation.** Similar to the definition of soil P saturation (DPS)[47], the
186 colloidal P saturation (DPS_{coll}) was used to assess the P adsorption capacity of soil colloids at 15
187 sites in Zhejiang Province. The DPS_{coll} was calculated using the following formula:

188 $$DPS_{coll} = \frac{P_{coll}}{Fe_{coll} + Al_{coll}} \times 100\%$$

189 where Fe_{coll}, Al_{coll}, and P_{coll} denote the Fe, Al, and P contents in the colloid, respectively, and the unit
190 of the Fe_{coll}, Al_{coll}, and P_{coll} is mmol kg^{-1}.

191 **Statistical Analysis.** Statistical analysis was performed using SPSS statistical software

package (SPSS Inc. Chicago, USA). A least significant difference (LSD) test at $P = 0.05$ level was used to determine significant differences among the results of different fertilization treatments. Origin 20.0 software (OriginLab Corp., US) was used for graphical processing. Pearson correlation analysis and linear fitting were used to identify the relationships between nano-, fine- and medium-colloidal P, and other colloidal and soil parameters.

■ RESULTS

Soil colloidal composition under varying agricultural geo-landscapes. The fractograms indicated that all 15 soil samples consisted of 2 or 3 size fractions as observed by the monitored elemental peaks with similar elution times between samples (Figure 1). The first peak contained the smaller nano colloids (approximately 0.6–25 nm; lower limit calculated from the membrane pore size of 1 kDa); the second peak was assigned to larger nano colloids and fine colloids (approximately 25–160 nm, fine colloidal fraction); the third peak (after release of the cross flow) was related to medium colloids (approximately 160–500 nm). In all H, all M and the L1 soils, all three fractions were observed, and generally dominated by the fine colloidal fraction except for L1. However, in L2–L5, only the first and third fraction was detected.

The soil colloidal P (0.6–500 nm) can be divided into three groups: high (H1–H5, 3583–6142 μg kg^{-1}), medium (M1–M5, 859–2612 μg kg^{-1}), and low (L1–L5, 514–653 μg kg^{-1}) (Figure 2, Table 1). The medium and high-level groups originated from the Basins and Mountains of Central and Southeast, which were characterized by very low P contents in the nano- and medium-colloidal size fractions, however, contained high amount of P in the fine-colloidal fraction. The fine-colloidal P content of the high-level group accounted for 77–89% of the total determined colloidal P content

(Figure 2, Table 1). Peaks of fractograms were much less clear for northern plains soils, which comprised the low-level colloidal P group (Figure 2). The fine- and medium-colloidal P here only accounted for 28–39% and 35–53%, respectively, of the total colloidal P measured (Table 1).

In general, C_{org}, Si, Al, and Fe were the main constituent elements of soil fine and medium colloidal fractions, while the C_{org} and Ca contents were most abundant of the monitored elements in the soil nano colloidal fraction (Figure S3, Table 2). When comparing different colloidal size fractions, the mean contents of C_{org}, Si, Al, and Fe (447, 164, 56.2 and 19.8 mg kg^{-1}, respectively), were much higher than the mean Ca concentration (1.8 mg kg^{-1}) in the fine and medium colloidal size (Figure S3, Table 2). However, in the nano colloidal fraction C_{org} and Ca were more abundant with mean contents of 150.7 and 0.7 mg kg^{-1} compared to the rather low mean contents of Fe and Al of 0.2 mg kg^{-1} and 0.5 mg kg^{-1}, respectively (Figure S3).

Linkages of colloidal P with organic carbon at regional scale. Colloidal Al, Fe, Ca, Si, and C_{org} compositions were significantly correlated with colloidal P ($P < 0.05$). However, the fitting results varied with the colloidal size fractions. For the nano colloidal fraction, only Ca and P displayed a linear relationship (Figure 3). However, linear relationships were found between P and C_{org}, Si, Al, Fe, and Ca in the fine colloidal fraction. In the medium colloidal fraction, there were linear relationships between P and C_{org}, Si, Al, and Fe, with the strongest relationship between P and Al (Figure 3).

The P saturation in the colloids showed that the L1–L5 had higher soil DPS_{coll} levels in the total fractions, with a mean of ca. 20%, whereas it was only 1% in H1–H5 and M1–M5 (Table 1). In

234 addition, our analysis showed that DPS_{coll} was closely related to colloidal C_{org} (Figure 4). When the
235 C_{org} concentration was < 200 mg kg^{-1}, the DPS_{coll} decreased sharply with the increase in the C_{org}
236 content, and when it exceeded 200 mg kg^{-1}, DPS_{coll} decreased gently with the increase of C_{org}, and
237 the scatter plot after log-transformation showed a negative correlation between C_{org} and DPS_{coll}
238 (Figure 4).
239
240 **Soil colloidal composition under different organic amendments input.** Carbon-based
241 fertilizer inputs did not affect the fractogram shapes of nano, fine, and medium colloids in the two
242 soils (Figures S4 and S5), and did not change the magnitude (medium level) of the colloidal P
243 distribution at the regional scale. The above assigned three size fractions were present in all
244 treatments. However, compared with the control, all treatments showed a (slight) increase in the
245 peak value (concentration), except for OFSB in which it decreased. Compared with CF, the OF
246 treatment mainly caused a significant increase of the C_{org} content of the nano colloidal fraction, but
247 Al, Fe, and Ca contents decreased in the fine colloids (Table S9, Figure S6). The BS treatment
248 significantly increased the C_{org} concentration in both nano and fine colloidal fractions ($P < 0.05$).
249 Furthermore, OFBS and BSSB significantly decreased the Al, Fe, and Ca contents in colloidal
250 fractions ($P < 0.05$).
251 Compared with CF at Site 1, the OF and OFSB treatments reduced the soil colloidal P content
252 (0.6–500 nm) by 33% and 43%, respectively, while at Site 2, BS increased colloidal P by 30% and
253 BSSB reduced colloidal P by 45% (Figure 5). In comparison with CF, the treatments of OF, OFBS,
254 and BSSB significantly reduced the concentration of fine colloidal P ($P < 0.05$), but had little effect

255 on nano colloidal P. However, the nano-colloidal P concentration of BS treatment was 50% higher
256 than that of CF, while the fine-colloidal P concentration of BS were 74%, 39%, and 178% higher
257 than those of CK, CF, and BSSB, respectively. In the medium colloidal fraction, no significant
258 differences of P content were detected between CK, CF, and OF/BS, however, in OFBS and BSSB
259 treatments, it was significantly lower than all other treatments. The truly dissolved P content of the
260 two soils significantly decreased after organic fertilizers application when compared to CF ($P <$
261 0.05), while the ratio between fine colloidal P and the truly dissolved P was higher in the two soils
262 with modified biochar (Figure S7).

263 ## ■ DISCUSSION

264 **Colloidal P formation as revealed by regional-scaled observation.** In this study, 2 or 3
265 colloidal fractions were found in 15 soils, which is similar to the findings in other soils or
266 streams[20,26,43]. However，we found two distinct linkages of organic matter with the nano-colloidal
267 P and fine- and medium-colloidal P, respectively. Nano colloidal P is correlated with organic carbon
268 and Ca and most likely forming a complex of C_{org}-Ca-P, while fine colloidal and medium colloidal
269 P is correlated with clay mineral elements and likely to form a complex of C_{org}-clay-P.

270 Colloids (< 450 nm) extracted from soil or stream water usually contain nano-sized organic
271 matter (complex organic acids) in the size range of 1–5 nm[19]. Moreover, some macromolecular
272 degradation products and excretes (<100 nm) by microorganisms within soil, mainly comprising of
273 protein, DNA, and fat as well as extracellular polymeric substance (EPS), co-exist with P, which
274 was revealed by the high concentrations of P-monoesters and P-diesters in the WDC extract
275 identified by NMR (Figure S8, Table S10). In most soils, nano-colloidal Fe and Al were present at

276 non-detectable levels, while Ca was present in significant amount along with organic carbon. This
277 is different from the finding[19] by Jiang et al, 2015 that implied the binding mechanism of organic
278 matter and amorphous Fe or Al oxides in the nano colloidal fraction (<20 nm) of an acidic soil.
279 However, a recent study showed that binary humic acid (HA)-Ca complexes could incorporate P by
280 forming ternary HA-Ca-P complexes[48], and the P may also be bound in nano-colloidal minerals of
281 apatite[49,50]. Furthermore, in calcareous soils, Ca^{2+} ions presumably bridged organic P and nano
282 colloidal negatively charged organic substances with carboxyl or phenolic groups[42,51,52]. Therefore,
283 we assume that nano colloids are dominated by organic compounds, and that their small-sized acid
284 moieties may form stable nanoparticles through the bridging of Ca^{2+} ions. This is also supported by
285 a strongly positive correlation with nano-colloidal Ca and P, but non-significant correlations with
286 nano-colloidal Fe, Al, or Si (Figure 3). However, the fact that there were little differences between
287 the C_{org} content in nano colloids of the soils, and C_{org}/P and Ca/P were basically fixed at
288 approximately 4000:1 and 4:1, respectively (Table 2 and S10), may indicate that the binding capacity
289 of P to nano colloids was limited.

290 The fine colloidal fraction with the highest intensity of the three separated size fractions in most
291 soils (H1–H5 and M1–M5) contains large amounts of C_{org}, Si, Al, and Fe, and this size fraction is
292 usually formed from stable organic-inorganic composite colloids consisting of clay minerals, Fe/Al
293 (hydr)oxides and organic matter[19,53]. This was revealed by the fixed molar ratio of Si/Al (1:0.5)
294 elements in the colloids (Table S11), which was consistent with the composition of 2:1 clay
295 minerals[19,54,55], and the fact that the soil clay minerals in the Zhejiang area contains more muscovite
296 and montmorillonite (Figure S9)[56]. Considering the relatively constant Fe/Si molar ratio

297 (approximately 0.1:1; Table S11), the clay minerals in this area may have the fixed ratio of
298 isomorphic substitution between Fe and Al[57], and/or Fe oxides form complexes with clay minerals
299 and organic matter, respectively. The medium colloidal fraction with a lower intensity, represented
300 the larger WDC, but may have included small aggregates of nano and fine colloids[19] and occasional
301 residual particles of sizes > 500 nm. In contrast to H1–H5 and M1–M5 soils, the fine colloidal
302 fraction of L1–L5 soils is missing, which may be related to the lower clay content of these soils
303 (Table S1). Some studies reported that the soil colloid content is closely related to the clay mineral
304 content[58,59], which presumably immobilizes the P in the soil through adsorption, ligand exchange,
305 and precipitation[19,60–61]. The fine-colloidal P dominated by C_{org}, Si, Fe, and Al, accounted for 80%
306 of the total colloidal P for H1–H5 and M1–M5 and 36% of total colloidal P for L1–L5. Note, Ca^{2+}
307 may also participate in the fine colloid formation process, connecting colloids and organic matter[42,62].
308 In addition, Ca^{2+} may play an important role in bridging inorganic P and organic P on clay minerals
309 accompanied by organic matter[45].

310 The P saturation in the colloids showed that the L1–L5 had significantly higher soil DPS_{coll}
311 levels than H1–H5 and M1–M5 (Table 1). Furthermore, there was a negative correlation between
312 the DPS_{coll} and C_{org} concentrations (Figure 4). Some studies also confirmed that when the surface
313 adsorption sites of soil are occupied by a large amount of organic substances, the adsorption capacity
314 of clay particles for phosphate ions will be weakened[63–64]. The organic carbon compounds with
315 competitive inhibition of P sorption are usually derived from low molecular weight organic acids
316 (LOAs), humic and fulvic acids, and organic leachates[65]. Therefore, the degree of P saturation in
317 fine and medium colloids can directly reflect the amount of soil P carried by the colloids which can

be used in the future to predict the P retention capacity of soil colloids and the risk of P loss. It is worth noting that in nano colloids fraction, the adsorption mechanism of P was not related to Fe and Al, but rather to the complexation of Ca and organic matter. Therefore, DPS_{coll} cannot be simply used for the prediction of nano-colloidal P in the soils studied.

Colloidal P in the fractograms of the agricultural soil colloids revealed spatial distribution characteristics at the regional scale (Table 1). This also may be subject to the differences in soil mineralogy and organic C storage. A study in the subtropical forests found that soil organic C was highly correlated with P sorption index[66], and many organic P compounds sorb strongly to positively charged mineral surfaces of amorphous Al and Fe oxide and the edge of clay minerals[67]. The subtropical monsoon affected humid climate zone in the middle and southern Zhejiang where the soil is rich in iron and aluminum and lower in C storage (Table S1)[56], resulting in the existence of a high-medium level group of colloidal P. Soils on the Northern Plain usually have lower aluminization and obvious accretion layers[56] and show a higher C storage and clay content (Table S1), resulting in the low level group of colloidal P. This pattern assignment also basically proves our first hypothesis that specific distribution patterns of colloidal P occur at regional scale. However, in fact, the terrain topography in Zhejiang Province is complicated[56], and the current classification of colloidal P fractions in this region is therefore still relatively coarse. Note, in view of the high instrumental and time effort of the AF4 technology with ICP-MS detection for testing a large number of samples, an initial set of 15 samples (3 repetitions each) was investigated in detail in this study to achieve a basic characterization at the regional scale. A further study in the future is needed to increase the sampling site density and evaluate the colloidal P distribution pattern in higher spatial

339 and possibly even temporal resolution at the regional scale.

340

341 **Effects of organic fertilizer inputs on soil colloidal P.** Our study found that 2 years of
342 differential fertilization had no significant effect on the fractogram shape (i.e., magnitude and
343 composition) of nano- and fine-colloidal sized particles isolated from two soils. Therefore, we
344 conclude that the soil textures and the contents of the particulate matrix elements (i.e., C_{org}, Fe, Al,
345 Si, Ca) affect the composition, distribution, and morphology of soil colloidal fractions, while the
346 difference in the type of fertilization and carbon inputs affects the concentration of compounds (i.e.,
347 P) adsorbed by colloids. However, the ability of colloids in different soils to carry P has been
348 determined by soil conditions, which is supported by the concept of DPS_{coll} proposed above.
349 Therefore, the first step to investigate the colloidal loss potential of a special type of soil is to
350 understand its organic and mineral phase composition. This is an important prerequisite for
351 formulating a P management strategy. Understanding this premise is of great significance to the
352 management of P loss in the agricultural field at different regional scale.

353 Indeed, fertilization regimes significantly affected the soil nano-colloidal P and fine-colloidal
354 P concentrations in a particular type of soil. Specifically, compared with the control, the application
355 of chemical fertilizer enhanced the P saturation of fine colloids by increasing the phosphate
356 concentration, and boosted the nano-colloidal and fine-colloidal P contents in the soil. The strong
357 correlation between the truly dissolved P or available P and colloidal P further confirmed the
358 occurrence of that process (Figure S10). Compared with CF, when organic fertilizer replaced 30%
359 of the chemical P, the colloidal P content significantly decreased. This was mainly due to the increase

of organic matter, which sheltered the colloidal adsorption sites[63,64], and limited the chances of the phosphate ions binding to colloids through adsorption, resulting in the reduction of the amount of colloidal P. We further found that compared with the application of chemical fertilizers, organic fertilizer and biochar applications increased the concentration of C_{org} in the soil colloids, but decreased the concentrations of Fe and Al (Table S10). A study of a loam soil with long-term application of poultry manure found that P was more likely to be associated with Ca than Fe or Al[68] and resulted in the conversion of relatively soluble Ca-P into more colloidal crystal phases. However, when applying biogas slurry instead of 30% chemical P, the amounts of nano-colloidal and fine colloidal P in the soil increased significantly (Figure 5). This may be due to the biogas slurry itself containing a large amount of organic colloidal P, which is bound to clay colloids or exist in the nano P form once entering the soil[29]. Compared with CF, OF, and BS, the application of modified biochar significantly reduced the amount of colloidal P in the soil. The two explanations for this are: 1) P adsorption on biochar[33], and 2) the modified biochar promotes the formation of soil aggregates[69]. Overall, more systematic field experiments including runoff and leaching losses are warranted to investigate and verify the effect of fertilization on the morphology and migration of colloidal P.

■ ASSOCIATED CONTENT

Supporting Information

Sections S1-S3; Figures S1–S10; Tables S1–S11

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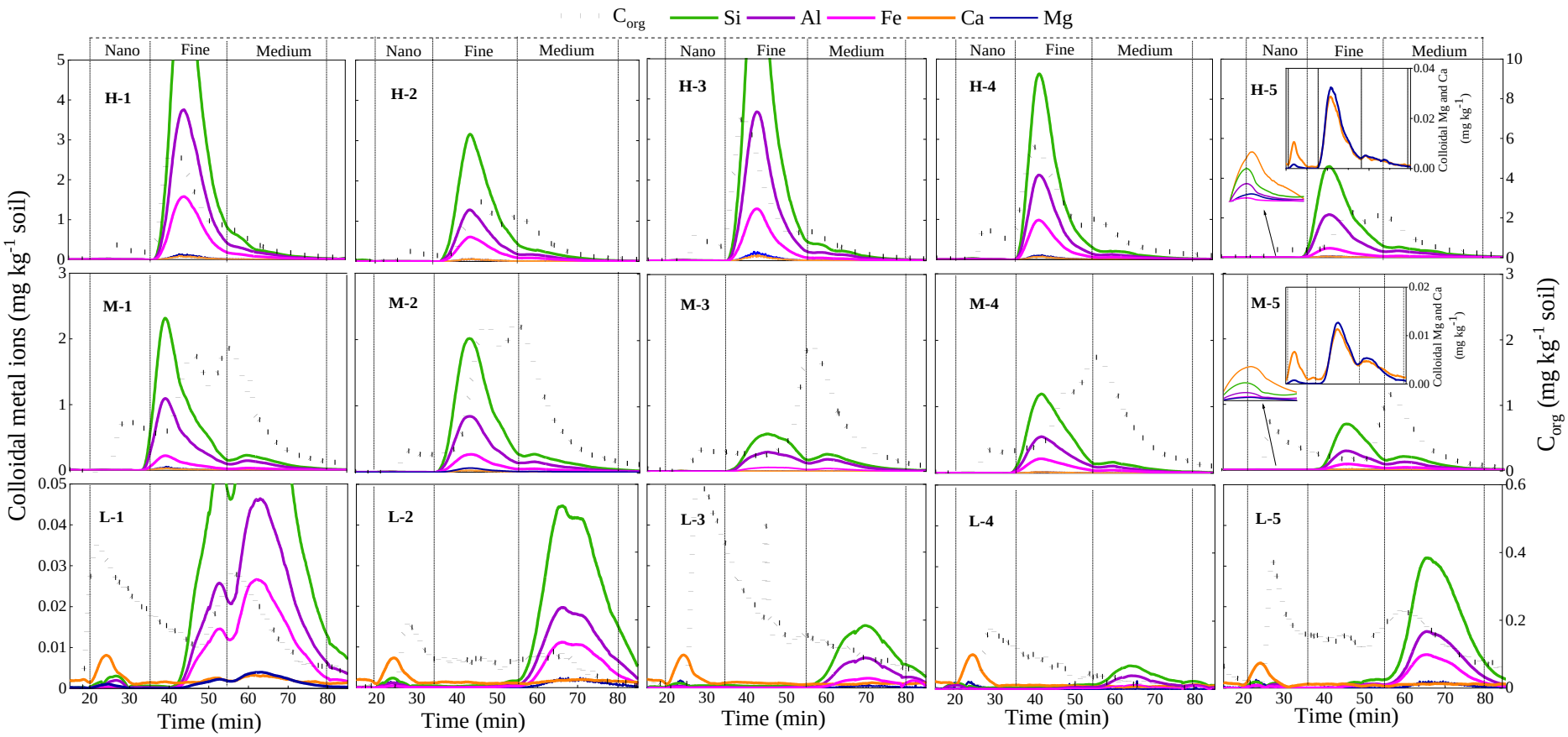

Figure 1. The fractogram of colloidal elements such as aluminum (Al), iron (Fe), silicon (Si), calcium (Ca), and organic carbon (C_{org}) in the soils at 15 sites by Asymmetrical Flow Field Flow Fractionation (AF4) coupled with Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) and Organic Carbon Detector (OCD). "Nano" is nano colloidal fraction (0.6–25 nm); "Fine" is fine colloidal fraction (25–160 nm); "Medium" is medium colloidal fraction (160–500 nm).

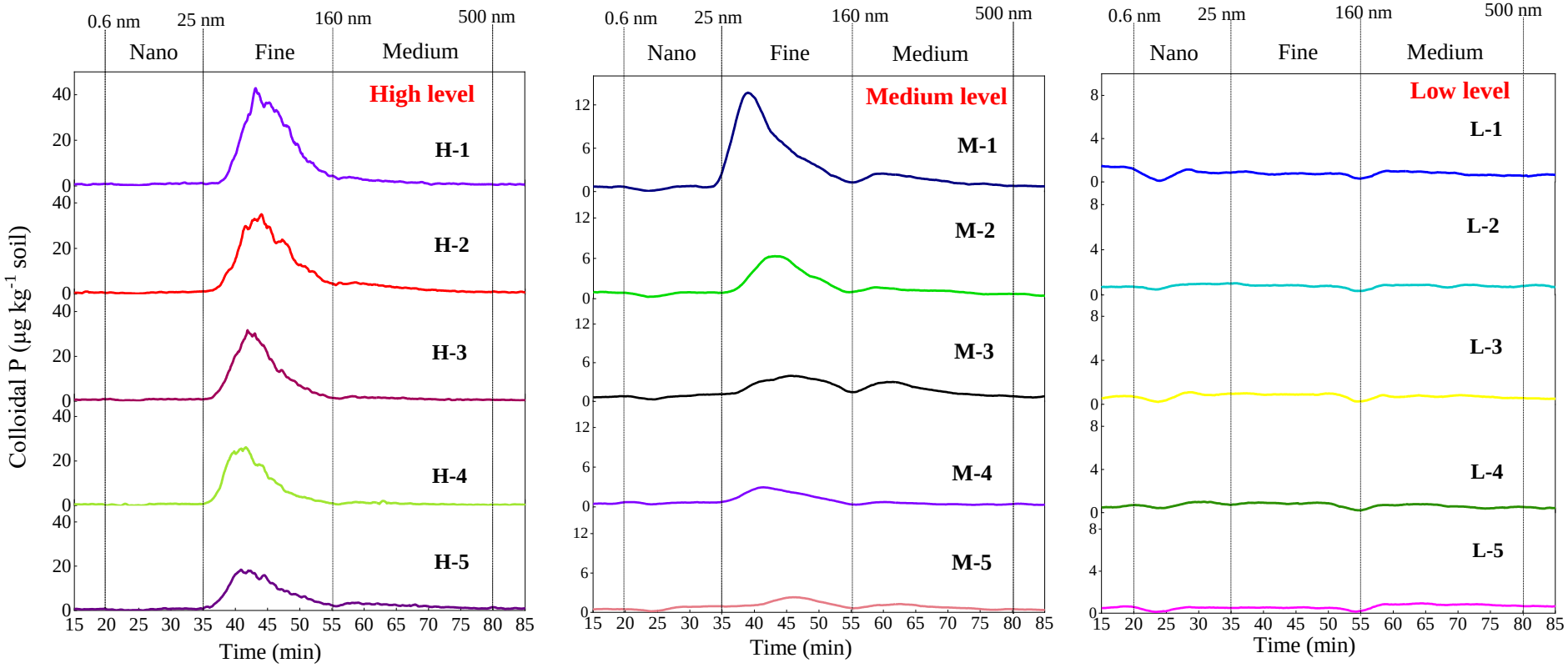

Figure 2. The fractogram of nano-colloidal (Nano), fine-colloidal (Fine) and medium-colloidal (Medium) phosphorus (P) in the 15 soils measured by Asymmetrical Flow Field Flow Fractionation (AF4) coupled with Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). High, medium, and low levels indicate the peak level of colloidal P in the soils. Y-axis are scaled differently for the three figures.

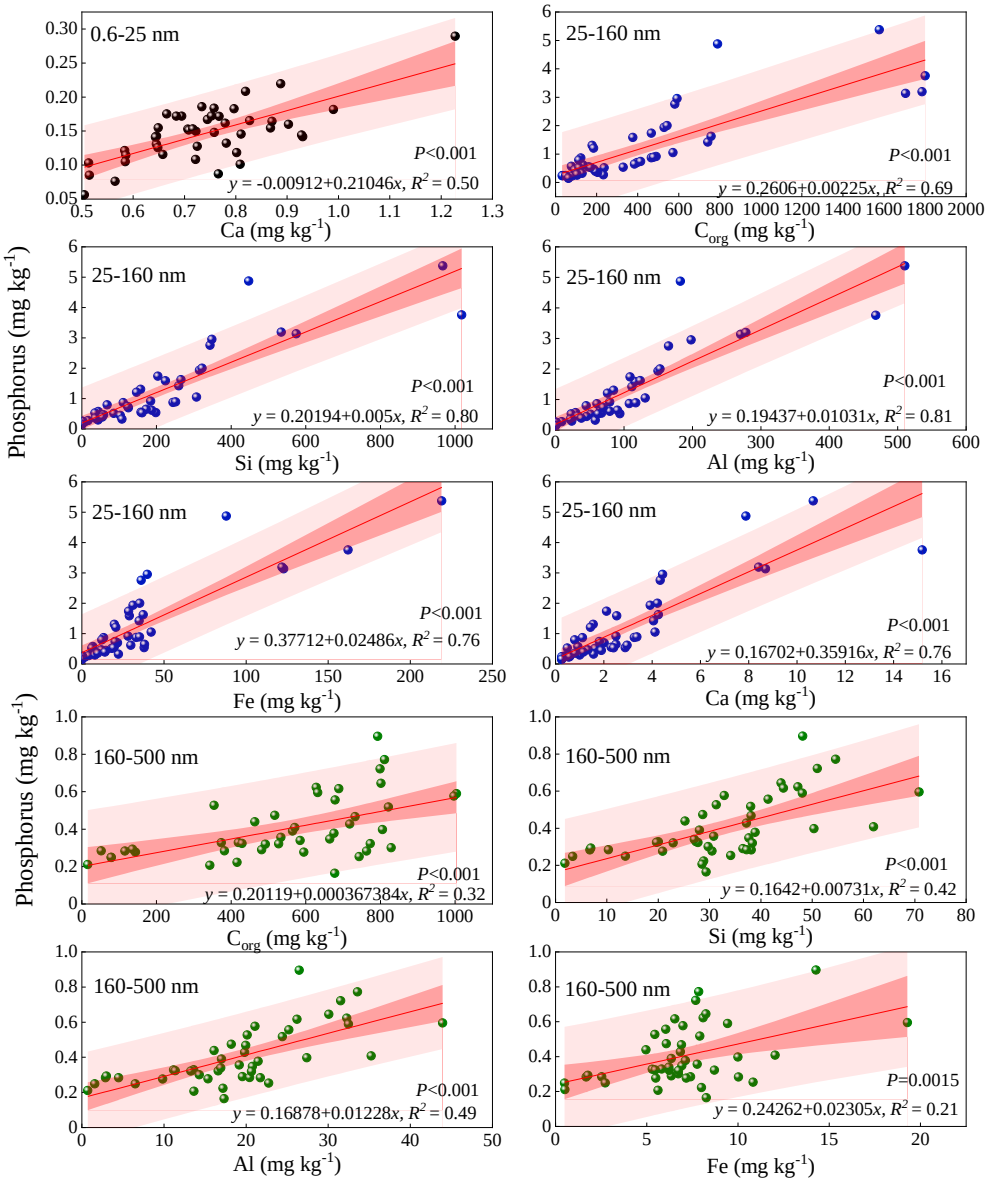

Figure 3. Nano- (0.6–25 nm), fine- (25–160 nm) and medium- (160–500 nm) colloidal phosphorus as a function of the content of other components in the same particle size fractions such as organic carbon (C_{org}), silicon (Si), aluminum (Al), iron (Fe), and calcium (Ca) (n=45)

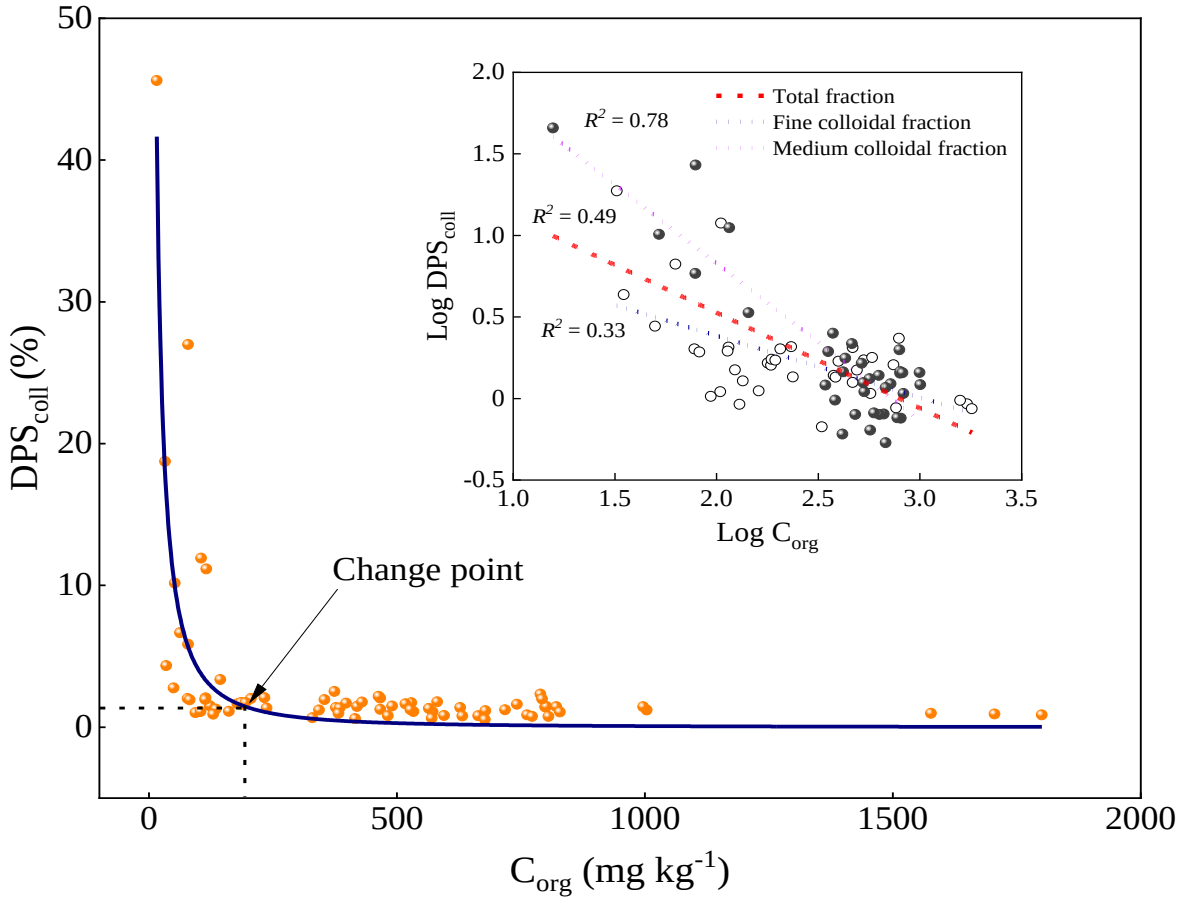

Figure 4. The relationship between colloidal phosphorus saturation (DPS_{coll}) and colloidal organic carbon (C_{org}) (a), and the related linear fitting plot of DPS_{coll} and C_{org} after Log transformation (b).

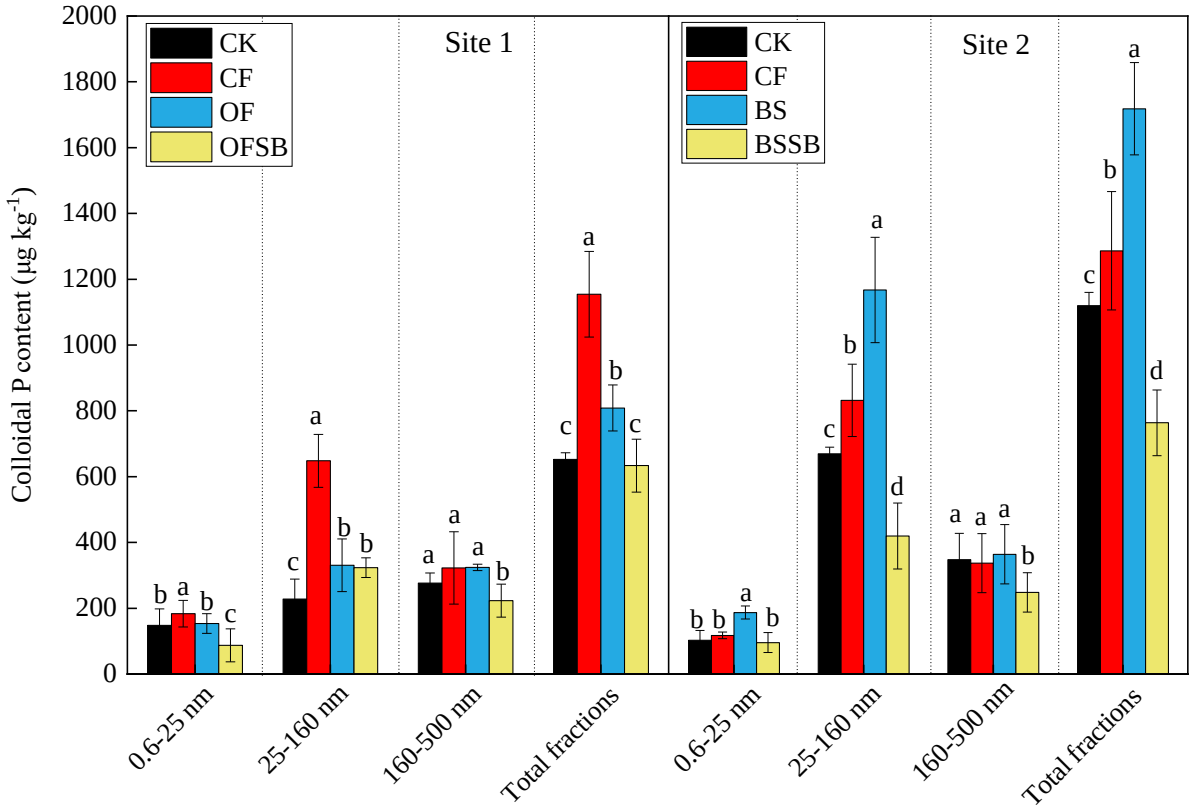

Figure 5. Nano-, fine- and medium-colloidal phosphorus (P) content of different fractions in the soils at Site 1 and Site 2 under different fertilization treatments (n=3). CK represents control without fertilizer; CF represents conventional fertilization (chemical fertilizer); OF or BS represents organic fertilizer (sheep manure) or biogas slurry instead of 30% P application rate of chemical fertilizer, respectively; OFSB or BSSB represents organic fertilizer or biogas slurry + modified rice straw biochar instead of 30% P application rate of chemical fertilizer, respectively; Lowercase indicated significant difference between different fertilization treatments at $P < 0.05$ level.

Table 1. Colloidal phosphorus content and saturation (DPS_{coll}) of different particle size fractions in the 15 representative soils with different land-use types in Zhejiang Province, China.

Soils	Colloidal P content (μg kg^{-1})				DPS_{coll} (%)		
	0.6–25 nm	25–160 nm	160–500 nm	Sum of fractions	25–160 nm	160–500 nm	Sum of fractions
H-1	172±11	5375±195	595±67	6142±214	0.8±0.1	1.0±0.1	0.8±0.2
H-2	142±25	4874±368	896±77	5912±359	1.9±0.3	2.4±0.1	2.0±0.2
H-3	155±13	3756±411	408±37	4318±498	0.6±0.1	0.9±0.1	0.6±0.3
H-4	103±9.0	3135±165	283±33	3603±189	0.8±0.1	0.9±0.1	0.9±0.2
H-5	108±10	2753±123	722±92	3583±189	1.3±0.0	2.0±0.1	1.4±0.2
M-1	121±11	1935±110	556±149	2612±99	1.0±0.1	1.7±0.2	1.2±0.1
M-2	150±24	1050±127	397±45	1597±145	0.6±0.0	1.1±0.3	0.8±0.2
M-3	160±29	863±102	623±77	1646±212	1.1±0.2	1.5±0.2	1.4±0.3
M-4	126±14	534±46	164±28	824±56	0.4±0.2	0.7±0.1	0.5±0.1
M-5	131±20	444±44	284±29	859±89	0.7±0.2	1.0±0.1	1.0±0.2
L-1	148±25	228±21	277±31	653±68	4.7±1.0	2.0±0.6	3.4±0.1
L-2	172±36	242±20	284±20	697±77	125±14	4.3±0.2	10±1.5
L-3	151±10	258±20	248±13	658±51	88±14	12±2.3	27±4.3
L-4	153±8.2	236±11	211±22	601±40	150±21	19±3.1	46±7.2
L-5	85±7.1	146±26	283±44	514±68	60±5.5	6.6±1.1	11±2.1

Table 2. The average (n=3) content of colloidal aluminum (Al), iron (Fe), silicon (Si), calcium (Ca), and organic carbon (C_{org}) content in the nano- (0.6–25 nm), fine- (25–160 nm), and medium- (160–500 nm) colloidal fractions of 15 soils determined by AF4-ICP-MS and AF4-OCD.

Sites	**0.6–25 nm (mg kg^{-1})**						**25–160 nm (mg kg^{-1})**						**160–500 nm (mg kg^{-1})**					
	C_{org}	Mg	Al	Si	Ca	Fe	C_{org}	Mg	Al	Si	Ca	Fe	C_{org}	Mg	Al	Si	Ca	Fe
H-1	166	0.10	0.75	1.2	0.69	0.31	1577	15	510	967	11	219	632	1.3	44	71	1.3	19
H-2	126	0.10	0.30	0.65	0.65	0.13	790	7.5	183	447	7.9	88	793	1.2	26	48	1.5	14
H-3	422	0.10	0.78	1.3	0.87	0.25	1800	23	468	1018	15	162	570	1.6	35	62	1.6	12
H-4	461	0.17	1.4	2.4	0.51	0.59	1705	12	271	574	8.7	123	763	1.0	22	38	1.0	10
H-5	123	0.09	0.24	0.49	0.72	0.06	582	4.9	165	343	4.3	36	799	1.0	32	51	1.0	7.7
M-1	218	0.14	1.7	3.1	0.58	0.42	529	5.5	150	315	3.9	31	679	1.0	25	41	0.77	6.0
M-2	97	0.08	0.20	0.46	0.72	0.07	572	9.2	131	307	4.1	42	806	2.0	27	50	1.0	10
M-3	92	0.12	0.15	0.31	0.90	0.04	123	1.1	61	111	1.1	13	628	0.7	32	47	0.78	8.1
M-4	103	0.07	0.30	0.57	0.65	0.12	330	2.9	94	198	2.9	38	679	0.62	17	29	0.84	8.3
M-5	161	0.07	0.15	0.36	0.65	0.06	94	2.0	45	103	1.9	15	382	1.0	20	37	1.1	7.4
L-1	112	0.08	0.16	0.29	0.76	0.04	82	0.30	3.3	7.4	0.55	1.9	144	0.83	9.9	21	0.79	5.5
L-2	42	0.07	0.09	0.21	0.68	0.04	35	0.02	0.14	0.36	0.38	0.06	52	0.46	4.5	10	0.57	2.5
L-3	165	0.09	0.04	0.15	0.71	0.02	105	0.03	0.24	0.45	0.37	0.04	80	0.11	1.6	3.4	0.36	0.50
L-4	44	0.09	0.02	0.09	0.72	0.01	32	0.01	0.12	0.29	0.26	0.04	16	0.02	0.73	1.8	0.24	0.52
L-5	78	0.09	0.06	0.12	0.51	0.07	63	0.03	0.15	0.30	0.25	0.13	116	0.28	2.9	6.7	0.34	1.7