https://doi.org/10.17221/140/2019-SWR

Organic acids promote phosphorus release from Mollisols with different organic matter contents

*Xiaoyan Yang[1], Chuandong Zhang[2], Haiping Gu[1], Xiangwei Chen[3], Erhui Guo[1]**

[1]School of Forestry, Henan Agricultural University, Zhengzhou, P.R. China
[2]The Third Geological Exploration Institute, Henan Bureau of Geo-Exploration Mineral Development, Zhengzhou, P.R. China
[3]School of Forestry, Northeast Forestry University, Harbin, P.R. China

**Corresponding author: guoerhui@126.com*

Citation: Yang X., Zhang C., Gu H., Chen X., Guo E. (2021): Organic acids promote phosphorus release from Mollisols with different organic matter contents. Soil & Water Res., 16: 59–66.

Abstract: Organic acids could improve the phosphorus (P) availability through enhancing the release of inorganic phosphorus (P_i) in the soil. However, the effects of organic acids on the P_i release are still poorly understood, especially from soils with different organic matter contents. Here, a biochemically produced humic acid and P fertiliser were added to the soil to modify the content of the soil organic matter (SOM) and soil P, respectively. And then the soil samples were incubated at 25 °C for 30 days. The release of P_i fractions (such as H_2O-P_i, $NaHCO_3$-P_i, NaOH-P_i, HCl-P_i, and Residual-P) from the soils with different organic matter contents in the presence of citric, oxalic, and malic acids was evaluated using a sequential chemical fractionation method. The results showed that the release of the $NaHCO_3$-P_i, NaOH-P_i, and HCl-P_i fractions also showed a decreasing trend with an increasing content of soil organic matter, and more NaOH-P_i than the other P_i fractions was generally released in the presence of organic acids. Considering the types of organic acids, oxalic acid and malic acid most effectively and least effectively released P_i, respectively. The path analysis indicated that the NaOH-P_i release had the highest direct and indirect effects on the total inorganic P (TP_i) release. NaOH-P_i was, therefore, the most effective source of P_i in the Mollisols.

Keywords: available phosphorus; black soil; correlation analysis; path analysis; phosphorus fractions

Phosphorus (P) is an essential nutrient required by crops. P deficiencies are relatively more common in the early part of the growing season (Hopkins et al. 2008), and, therefore, can limit crop growth in a wide range of soils across the world (Holford 1997). Phosphate fertilisers are applied to soils to maintain and improve crop yields (Romanyà et al. 2017). However, excessive P fertiliser applications cause P to accumulate in the soil (Ahmed et al. 2019) and cause various other environmental problems (Daniel et al. 1998). P is present in the soil in various forms that have a different availability and solubility. A sequential P fractionation scheme based on a method developed by Hedley et al. (1982) has been used in many studies of soil P fractions (Rakotoson et al. 2015; Yan et al. 2017; Yang et al. 2019a). This method allows one to determine the P availability in soils associated with vegetation (DeBruler et al. 2019; Liao et al. 2020), management regimes (Shi et al. 2015), and land use change (Sun et al. 2019). Among them, inorganic P (P_i) in the soil is the preferred source of P for plants and may pose greater risks for eutrophication than organic P (Zhang et al. 2018), because the potential bioavailability of

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This work was supported by National Natural Science Foundation of China (No. 41701360).

https://doi.org/10.17221/140/2019-SWR

organic phosphorus in the various fractions lacks experimental validation (Turner et al. 2005). So, it is important to study soil P_i fractions to improve our understanding of soil P dynamics.

Organic acids exuded by plants and soil microbiota (Romanyà et al. 2017) can transform insoluble P into plant-available forms and, therefore, affect the P bioavailability (Wang et al. 2018; Yang et al. 2019a). However, the abilities of organic acids to solubilise P are dependent on the soil type, the soil organic matter (SOM) content, and the structures and concentrations of the organic acids themselves (Oral & Uygur 2018). SOM is one of the important constituents in the soil in terms of maintaining the solubility of P in the soil (Yan et al. 2016; Romanyà et al. 2017). The effects of SOM on the soil P availability have been widely reported with contradictory results (Guan et al. 2006; Hua et al. 2008; Clarholm et al. 2015). In addition, the release of organic acids destabilises the organic matter in organomineral complexes and causes decomposition that affects the effectiveness of the SOM onto the P bioavailability (Clarholm et al. 2015). It is, therefore, important to understand the effects of the interactions between the organic acids and the SOM on the availability of P to ensure that phosphate fertilisers are used effectively. Few studies on this have been performed.

Therefore, the main aim was to identify the roles organic acids play in releasing P from different P_i fractions in Mollisols with different SOM contents which were prepared by adding humic acids to the natural soil. The contribution of the different P_i fractions to the release of the total P_i (TP_i) was assessed by performing a path analysis.

We hypothesised that different types of organic acids, due to the different affinities for the dominant bridge-forming polyvalent metal cations in the SOM, as well as the suitable size and sufficient lipophilicity to penetrate hydrophobic SOM structures (Piccolo 2002), promoted different levels of P release in soils with different SOM contents. In addition, the effect of the individual P_i fraction on the release of the total P_i varied, and this variation, to some extent, depended on its content and bioavailability.

MATERIAL AND METHODS

Soil sampling

The experiments were performed using soil from Keshan Farm, which is in a typical black soil area in northeast China (N 48°12'–48°23', E 125°08'–125°37'). The soil was classified as a Mollisol. Soil samples from 40–45 cm deep were collected from five randomly assigned points on the arable land used for a seasonal maize crop to obtain a lower SOM content for the simulation experiment. The soil samples were dried and then passed through a 2 mm sieve before being used. The bulk density was measured using the core sampling method, using a core size of 100 cm^3 (Chen 2005). The soil pH was measured using a pH meter with a glass electrode in a 1 : 2.5 (w/v) soil-to-deionised water ratio (Chen 2005). The soil organic carbon content was determined using a Multi EA 400 elemental analyser (Vario EL cube, Elementar, Germany), and a conversion factor of 1.724 was used to calculate the SOM (Chen 2005). The soil sample was digested in a mixture of H_2SO_4 and $HClO_4$ (Parkinson & Allen 1975) to obtain the total P fraction. The available P was extracted with 0.03 mol/L NH_4F–0.025 mol/L HCl and determined using the colorimetric method developed by Murphy and Riley (1962). The soil bulk density was 1.17 g/cm^3, the pH was 6.18, the SOM content was 20.5 g/kg, the total P content was 442 mg per kg, and the available P content was 5.04 mg/kg.

Experimental design

Preparation of "spare" soil samples with different SOM contents. Biochemically produced humic acids purchased from Aladdin Biochemical Technology Co. (Shanghai, China) were added to the soil to modify the SOM content. The humic acids have a negligible total P content and a C content of 36.7%. Humic acids of 0%, 4%, 8%, 11% (humic acid/ soil sample (w/w)) were added to 300 g of soil to give samples with SOM contents of 20.5, 44.6, 68.9, and 99.6 g/kg, which were labelled SOM_1, SOM_2, SOM_3, and SOM_4, respectively. A KH_2PO_4 fertiliser at a rate of 200 mg P/kg of soil was then added to each soil to increase the P content (Jalali & Ranjbar 2010). Deionised water was added to adjust the water content to 70% of the field capacity and then replenished every other day using the gravimetric method during incubation. The samples were incubated at 25 °C for 30 days, because the contents of the P fractions in the soil samples treated with the KH_2PO_4 and humic acids were almost stable after being incubated for 30 days in the pre-experiment. Each treatment was undertaken in triplicate, thus, twelve soil samples were incubated. After incubation, the three replicate soils of the same treatment were thoroughly mixed, dried, and then were passed through a 2 mm sieve before use. These samples are called "spare" soil samples below, and there are four "spare" soil samples (namely, SOM_1, SOM_2, SOM_3, and SOM_4) in total.

"Spare" soil analysis. A sequential P fraction extraction was performed using a modified version of a fractionation scheme developed by Hedley et al. (1982). Four aliquots of each soil sample were subjected to the extraction procedure. A 0.5 g aliquot of a soil sample was placed in a 50 mL centrifuge tube and sequentially extracted with 30 mL of H_2O, 0.5 mol/L of $NaHCO_3$ (at pH 8.5), 0.1 mol/L of NaOH, and then 1 mol/L of HCl, for 16 h with each extractant. The soil residue was then digested in a mixture of H_2SO_4 and $HClO_4$ to obtain the P content of the solid residue (later labelled Residual-P) to be determined. The P concentrations in all the above-mentioned extracts were determined using the colorimetric method developed by Murphy and Riley (1962).

The soil pH, and the contents of the total P, available P, and SOM in the "spare" soil were determined as described above (see the part of the soil sampling).

Organic acid amendments. A 1.0 g aliquot of "spare" soil was extracted for 30 min with 20 mL of a solution containing oxalic acid, citric acid, or malic acid at a concentration of 2.5 mmol/L, respectively. These are the most important organic acids exuded by plants and the mycorrhizae (Clarholm et al. 2015). The organic acid concentration was selected taking the concentrations found in rhizosphere samples by Santos et al. (2017) into consideration. The soil suspension was centrifuged at 5000 rpm for 10 min and passed through a 0.45 μm membrane filter. The total inorganic P (TP_i) concentration in the supernatant was determined using the molybdenum blue colorimetric method (Murphy & Riley 1962). The amount of TP_i release was defined as the difference between the amount of TP_i extracted with and without the organic acids in the extractant solution (Yang et al. 2019a). Each soil sample that had been extracted with an organic acid was then washed with alcohol to remove the residual organic acid between the soil particles (Liu et al. 2009), then the P_i fractions were determined using the sequential extraction method modified by Hedley et al. (1982). This divided the P_i into the H_2O-P_i, $NaHCO_3$-P_i, NaOH-P_i, HCl-P_i, and Residual-P fractions. The amount of each P_i fraction release was defined as the difference between the P_i fraction concentration found when the soil had and had not been treated with the organic acids (Yang et al. 2019a). Each treatment was performed on four separate soil aliquots.

Statistical analyses

A one-way analysis of variance (ANOVA) was performed and the mean values were compared using *t*-tests ($P < 0.05$). Simple correlation analyses were performed to investigate the interactions between the release of TP_i and the release of the different P_i fractions. Path analyses were performed to attempt to explain the contributions of the release of the different P_i fractions from the TP_i. All the statistical analyses were performed using SPSS 16.0 (IBM, Armonk, NY, USA), and a significance threshold of $P < 0.05$ was used. The figures were drawn using SigmaPlot 10.0 (Systat Software, San Jose, CA, USA).

RESULTS

Properties of P in different soils. No significant differences were found between the TP contents within the soil samples with the different SOM contents ($P < 0.05$), indicating that the addition of humic acid did not affect the soil TP contents (Table 1). This indicated that the humic acid added to the soil samples contained almost no P. The available P contents increased as the SOM content increased.

Most of the P_i fractions were in the moderately labile NaOH-P_i and the most stable Residual-P frac-

Table 1. Soil pH and P contents in the soils with different organic matter contents

Soil samples	pH	TP	AP	P_i fractions (mg/kg)				
		(mg/kg)		H_2O-P_i	$NaHCO_3$-P_i	NaOH-P_i	HCl-P_i	Residual-P
SOM_1	6.14	616^a	81.32^c	41.84^a	76.08^a	121.76^c	42.67^b	134.21^a
SOM_2	5.80	621^a	84.77^{bc}	39.30^b	73.81^a	129.25^{bc}	43.75^b	131.29^a
SOM_3	5.58	623^a	86.83^{ab}	41.76^a	68.47^b	133.00^b	44.10^b	129.45^a
SOM_4	5.19	620^a	90.81^a	42.48^a	53.61^c	156.83^a	47.18^a	123.56^a

The different lowercase letters indicate a significance among the different soil organic matter (SOM) content soils at $P < 0.05$; TP – total phosphorus; AP – available phosphorus; SOM_1 – 20.5 g/kg; SOM_2 – 44.6 g/kg; SOM_3 – 68.9 g/kg; SOM_4 – 99.6 g/kg; P_i – inorganic phosphorus

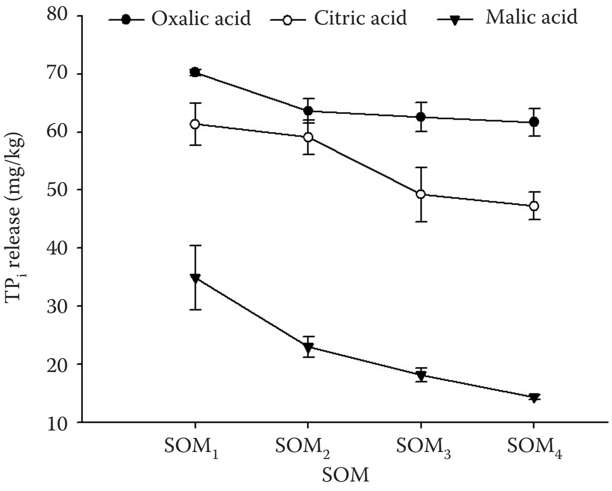

Figure 1. The amounts of total inorganic P (TP_i) release from the different organic matter content soils

SOM_1, SOM_2, SOM_3 and SOM_4 represent 20.5, 44.6, 68.9, and 99.6 g/kg, respectively

tion. As the SOM content increased, the $NaHCO_3$-P_i content decreased gradually and it was higher for SOM_1 than for SOM_2–SOM_4. In contrast to the $NaHCO_3$-P_i content, the NaOH-P_i and HCl-P_i content increased as the SOM content increased, while the H_2O-P_i and Residual-P content fluctuated as the SOM content varied.

TP_i release. The organic acids could solubilise the TP_i significantly (Figure 1). When the SOM content was the same, more TP_i was released from all four soil samples by the oxalic acid compared to the other acids. Citric acid released the second largest amount of TP_i and malic acid released the least. Significant differences were found between the amounts of TP_i released by the three organic acids from all the soil samples ($P < 0.05$). This indicated that citric acid was more effective in releasing the TP_i than both the citric and malic acids.

By the same organic acid, the amount of TP_i release decreased significantly as the SOM content increased ($P < 0.05$). The degree to which the amount of the TP_i release decreased as the SOM content increased was lowest for the oxalic acid, the next lowest was for the citric acid, and highest for the malic acid. This revealed that the ability of malic acid to release the TP_i was more affected by the organic matter content than that of the oxalic and citric acid.

Release of different P_i fractions. Table 2 shows that more NaOH-P_i than the other fractions was released except for with malic acid, which enhanced the release of $NaHCO_3$-P_i more than the other fractions, followed by $NaHCO_3$-P_i, then HCl-P_i, and finally Residual-P.

At the same SOM content, the amount of the H_2O-P_i release had a negative value, indicating that the H_2O-P_i content was higher in the presence of organic acids. In contrast, the amounts of the $NaHCO_3$-P_i, NaOH-P_i, and HCl-P_i released by the organic acids had positive values, and were the highest for oxalic acid, the next highest for citric acid, and the lowest for malic

Table 2. The amounts of the inorganic phosphorus (P_i) fractions released by the organic acids (in mg/kg)

Soil samples	Organic acids types	H_2O-P_i	$NaHCO_3$-P_i	NaOH-P_i	HCl-P_i	Residual-P
SOM_1	oxalic acid	-17.61^{aB}	39.15^{aA}	72.96^{aA}	10.10^{aA}	5.67^{aA}
	citric acid	-12.55^{bC}	29.23^{bA}	60.59^{bA}	8.44^{bA}	5.58^{aA}
	malic acid	-6.10^{cC}	21.36^{cA}	18.33^{cA}	2.55^{cA}	4.92^{aA}
SOM_2	oxalic acid	-23.468^{aA}	28.18^{aB}	68.96^{aAC}	9.41^{aAB}	4.34^{aA}
	citric acid	-18.68^{bB}	24.26^{bB}	57.18^{bAB}	8.39^{bA}	4.03^{aA}
	malic acid	-8.48^{cB}	13.70^{cB}	13.70^{cB}	2.66^{cA}	3.11^{bA}
SOM_3	oxalic acid	-24.38^{aA}	20.38^{aC}	67.66^{aBC}	9.01^{aB}	3.64^{aA}
	citric acid	-23.95^{aA}	17.25^{bC}	56.41^{bAB}	7.80^{aA}	3.08^{aA}
	malic acid	-9.23^{bB}	13.33^{cB}	13.29^{cB}	2.24^{bA}	3.00^{aA}
SOM_4	oxalic acid	-26.53^{aA}	18.74^{aC}	64.00^{aB}	8.79^{aB}	3.08^{aA}
	citric acid	-23.33^{aA}	17.11^{bC}	52.53^{bB}	7.89^{aA}	3.36^{aA}
	malic acid	-12.93^{bA}	13.50^{cB}	9.71^{cB}	2.58^{bA}	2.05^{aA}

The different lowercase letters indicate a significance among the different organic acids types at $P < 0.05$; the different capital letters indicate a significance among the different soils of the soil organic matter (SOM) content at $P < 0.05$; SOM_1 – 20.5 g/kg; SOM_2 – 44.6 g/kg; SOM_3 – 68.9 g/kg; SOM_4 – 99.6 g/kg

Table 3. Correlation matrices between the release of the total inorganic P (TP$_i$) and the inorganic phosphorus (P$_i$) fractions

	TP$_i$	H$_2$O-P$_i$	NaHCO$_3$-P$_i$	NaOH-P$_i$	HCl-P$_i$	Residual-P
TP$_i$	1.000	–0.649**	0.747**	0.958**	0.917**	0.302*
H$_2$O-P$_i$		1.000	–0.147	–0.751**	–0.741**	0.052
NaHCO$_3$-P$_i$			1.000	0.664**	0.629**	0.427
NaOH-P$_i$				1.000	0.957**	0.218
HCl-P$_i$					1.000	0.166
Residual-P						1.000

*, **Significant at $P < 0.05$, 0.01

Table 4. Direct effects and indirect effects of the individual inorganic phosphorus (P$_i$) release on the total inorganic P (TP$_i$) release

	Direct-path coefficient	Indirect-path coefficient				
		H$_2$O-P$_i$	→NaHCO$_3$-P$_i$	→NaOH-P$_i$	→HCl-P$_i$	→Residual-P
H$_2$O-P$_i$	–0.002		–0.026	–0.590	–0.033	0.002
NaHCO$_3$-P$_i$	0.176*	0.000		0.522	0.028	0.020
NaOH-P$_i$	0.786**	0.002	0.117		0.043	0.010
HCl-P$_i$	0.045	0.002	0.111	0.752		0.008
Residual-P	0.048	0.000	0.075	0.171	0.007	

*, **Significant at $P < 0.05$. 0.01

acid. However, the amounts of the Residual-P release were not significantly different for the soil samples.

For the same organic acid, the absolute amount of the H$_2$O-P$_i$ release increased, while the amounts of the NaHCO$_3$-P$_i$, NaOH-P$_i$, and HCl-P$_i$ release decreased as the SOM content increased (but with different levels of significance). The amounts of the Residual-P release were not significantly different for the soil samples with the different SOM contents (Table 2).

Correlation analysis. The release of TP$_i$ positively correlated with that of the P$_i$ fractions ($P < 0.05$) (Table 3), and the NaOH-P$_i$ release made the strongest correlation. For different P$_i$ fractions, significant correlations were found between the release of the NaHCO$_3$-P$_i$, NaOH-P$_i$, and HCl-P$_i$. The release of the H$_2$O-P$_i$ was significantly negatively correlated with that of the NaOH-P$_i$ and HCl-P$_i$ ($P < 0.01$).

Path analysis. A path analysis was performed to assess the direct and indirect effects of the P$_i$ fractions release from the TP$_i$ (Table 4). The direct-path coefficients indicated that the release of the NaHCO$_3$-P$_i$ and NaOH-P$_i$ directly and significantly affected the TP$_i$ release ($P < 0.05$) and that the NaOH-P$_i$ release made a larger contribution to the TP$_i$ release ($P < 0.01$) than did the NaHCO$_3$-P$_i$ release. The indirect-path coefficients indicated that the indirect effects of the P$_i$ fractions release on the TP$_i$ release were stronger for the release of the NaOH-P$_i$ than that of the other fractions. The release of the H$_2$O-P$_i$, HCl-P$_i$, and Residual-P made small contributions to the TP$_i$ release.

DISCUSSION

In our study, the abilities of organic acids to release the P$_i$ decreased gradually as the SOM content increased. In a previous study of P adsorption in Mollisols, Yang et al. (2019b) found that the maximum P adsorption capacity and soil P adsorption index increased with the SOM content. This ultimately caused smaller amounts of P$_i$ to be released by the organic acids as the SOM content increased. Gerke and Hermann (1992) and Oburger et al. (2011) found that organic-inorganic complexes formed between the humus (part of the SOM) and the iron/aluminium species provided important sites for the adsorption of P$_i$, giving, for example, ternary humic-Al (Fe)-P$_i$ complexes. This would increase the amount of P that would be adsorbed and markedly decrease the P$_i$ concentrations in the soil solution. However, Summerhays et al. (2015) found that adding organic acids created conditions similar to the conditions found in the presence of SOM, minimising the P solubilisation from the soil cations. This was also found for alkaline/calcareous soils in which excess cations precipitate or adsorb labile P. In conclusion, the data suggested that the high organic acid contents of a soil with a high SOM content did not improve the P_i bioavailability to the extent offered by the high organic acid contents of the soil with a relatively low SOM content. Exogenous organic acids appeared to most effectively improve the release of P from the soil with a low to moderate SOM content. Kpomblekou et al. (2003) found that acids were more effective in releasing P from low and medium reactive phosphate rocks than from high reactive phosphate rocks. We used soils with high P contents through the addition of external P. Future studies should be performed using a soil with a low P content.

For the inorganic P fractions, the content of the NaOH-P_i in the "spare" soil samples and the amounts of the NaOH-P_i release in the presence of the organic acids were higher than those of the other P_i fractions, indicating that the NaOH-P_i fraction could be a source of P_i to the plants. The path analysis results indicated that release of the NaOH-P_i fraction had the strongest direct and indirect effects on the TP_i release. The simple correlation analysis results agreed with that result, the highest correlation coefficient being for the relationship between the release of the NaOH-P_i and the TP_i. This confirmed that the NaOH-P_i was a more effective source than the other fractions in the Mollisol at an intermediate time-scale. The strong direct effect of the $NaHCO_3$-P_i release on the TP_i release and the correlation coefficient between them indicated that the $NaHCO_3$-P_i could make a strong contribution to the plant-available P in agricultural soils in the short term because it was rapidly desorbed from the soil particle surfaces (Cross & Schlesinger 1995). Verma et al. (2005) found that the contributions of the $NaHCO_3$-P_i to the P availability to the plant uptake of P were higher for acidic soils that had been continually fertilised with mineral and organic fertilisers than for other soils. Yang et al. (2006) also found that the $NaHCO_3$-P_i and NaOH-P_i contributed more than the other P fractions to the available P (Bray P) in red soil regions. The lower direct-path and indirect-path coefficients for the release of the H_2O-P_i, HCl-P_i and Residual-P could probably be explained by the lower contents of the H_2O-P_i and HCl-P_i, and the smaller release of the HCl-P_i and Residual-P by the organic acids. These results were consistent with the moderately resistant HCl-P_i fraction and the highly resistant Residual-P fraction being less effective than the $NaHCO_3$-P_i and NaOH-P_i as sources of P_i to plants (Rose et al. 2010). In addition, a significant negative correlation was found between the release of the H_2O-P_i and NaOH-P_i. This explained the discovery by Tiessen et al. (1984) that most labile P originated in the NaOH-P_i fraction.

In our study, the high doses of humic acids, as well as P fertilisers, were chosen to explore the soil P release with different organic matter contents. The significant results are then obvious and these results have important theoretical implications for our understanding of the P release mechanism. Despite this, considering the lower amounts of P fertilisers and organic matter in agricultural practice, further work is needed to determine the effect of low doses of humic acids as well as P fertilisers under different gradients on the soil P release.

CONCLUSION

The moderately labile NaOH-P_i and stable Residual-P fraction contents were higher than the other P_i fraction contents of Mollisols with different SOM contents. The effects of the organic acids on the P_i release were related to the SOM contents. Oxalic, citric, and malic acids increased the release of the TP_i and the individual P_i fractions, and the abilities of the acids to release P decreased as the SOM content increased. In other words, the higher the SOM content, the weaker the abilities of the organic acids to release the different P_i fractions. For the P_i fractions, more NaOH-P_i than the other P_i fractions was released by the organic acids. The amounts of the $NaHCO_3$-P_i release were the second highest. The path analysis results indicated that the organic acids increased the availability of P mainly by releasing NaOH-P_i and $NaHCO_3$-P_i from the Mollisols.

Acknowledgement: We thank G. Thomas, Ph.D., from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

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Received: November 5, 2019
Accepted: September 10, 2020