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ORIGINAL PAPER

Modification of P availability by endogeic earthworms (Glossoscolecidae) in Ferralsols of the Malagasy Highlands

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Abstract Low phosphorus (P) availability in Ferralsols of the Malagasy Highlands is a major limitation to crop growth. Direct seeding mulch-based cropping practices which were adopted in the region to improve and sustain soil fertility are known to favour earthworms' presence. The mesocosm study aims to analyse the effect of an endogeic geophageous earthworm species on the soil P status. Total P content (P_t), NaOH-extractable P content, P ions (Pi) concentration (C_p) in solution and rapid and slow reactions of Pi in solution with solid phase were determined in two Malagasy Ferralsols. Both C_p and reactions rates were assessed in laboratory batch experiments using ^{32}Pi labelling and isotopic exchange kinetics (IEK). The P_t values were 836 and 349 mg P g^{-1} in a clayey soil and a sandy–clayey soil, respectively. For both soils, NaOH-extractable organic P was significantly higher in earthworm casts than in parent soils, whereas Pt was unchanged. Also, the effect of earthworm ingestion significantly changed parameters of the IEK. In casts compared with the soil from which they were derived, the immediate isotopically exchangeable Pi ($E_{1\ min}$) increased by 116%, whereas relative rates of Pi release at the solid-to-solution with time were slightly lowered. The effect of earthworm ingestion on IEK corresponded to a transfer of slowly exchangeable Pi towards quicker Pi pools of exchange. However, according to the literature, the increase in $E_{1\ min}$ remained below the critical level for optimal growth, stating that the soils remained P-deficient even in the presence of active and numerous earthworms.

Keywords Phosphorus · Bioavailability · ^{32}P isotopic dilution · Earthworm casts · Tropical soil

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Introduction

Low fertility of the *tanety* (upland) soils is a major constraint for sustaining the agricultural productivity of agroecosystems in the Highlands of Madagascar. These acidic Ferralsols (FAO 1998) have a considerable capacity to adsorb and fix phosphate ions. As a consequence, the plant-available soil P is naturally very low and limits crops growth and yield (Rabeharisoa 2004). Increasing P availability in such soils is therefore essential for improving agricultural productivity. Direct seeding mulch-based cropping (DMC) systems, suggested as an alternative for reducing soil erosion and improving soil fertility, were developed since the early 1990s

in Madagascar. These cropping systems are characterised by no-tillage and permanent soil cover either by organic residue mulch or living cover crop. Such practices enhance soil organic matter (SOM; Six et al. 2002; Razafimbelo et al. 2008), providing a long-term nutrient reserve for plants. These systems also enhance soil biological activities (Rabary et al. 2008), as SOM provides food and substrate for soil organisms ranging from macroinvertebrates to heterotrophic bacteria (Lavelle et al. 2001). Moreover, a significant proportion of total P is under organic forms, making biological processes vitally important for enhancing soil P availability to plants (Oberson et al. 2006). Several authors reported the potential of the soil biological component that includes bacteria, mycorrhiza and macrofauna to compensate P deficiencies in various agroecosystems (Oberson et al. 2006; Turner et al. 2006). Earthworms are among the most important soil macrofauna influencing the physical, chemical and biological properties of soils and may be highly influential in increasing soil P availability (Brossard et al. 1996; Chapuis-Lardy et al. 1998; Nziguheba and Bünneman 2005). In the Malagasy soils under DMC systems, earthworms' presence can average 120 individuals per square metre (Coq et al. 2007).

Many chemical extractions are used to assess soil P status and its availability to plants. Sodium hydroxide (NaOH)-extractable fraction comprises inorganic P forms that are considered as bioavailable in the short term besides more slowly available P, and it can provide an approximate measure of potentially available P (Fardeau 1996). Alternatively to chemical extractions, isotopic exchange kinetics (IEK) were developed to assess P ions (Pi) concentration in solution and both rapid and slow reactions rates of Pi in solution with solid constituents of soils (Fardeau et al. 1991; Fardeau 1993, 1996). The use of ^{32}Pi as a tracer of Pi in solution of soil suspensions at steady state provides a biogeochemical assessment of soil P availability. By contrast with chemical extractions in which P forms, and therefore processes, are not clearly identified and stated, the IEK quantify specific P forms and a defined process. The P forms are the P ions ($H_2PO_4^-$ and HPO_4^{2-}), which are the effective species absorbed by roots of growing plants. The process accounted for the replenishment of Pi in solution is the diffusion at the solid-to-solution interface. Plant roots remove Pi from the soil solution, inducing a concentration gradient which is the driving force of the Pi transfer between liquid and solid phases of soil. The IEK method gives a set of experimental data of phosphate concentration in soil solution (C_p) and gross amounts of diffusive Pi over short periods. This set is then used to calibrate mathematical equation and calculate both dissolved and diffusive Pi for much longer periods. This approach has been extensively used to specifically assess Pi availability in a variety of soils (Morel et al. 1994; Sinaj et al. 2001).

The main objective of the present mesocosm study developed in two Malagasy Ferralsols was to investigate and quantitatively analyse the effects of earthworm activity on the soil P status, especially on its availability to plants. Soil P status was assessed by total P, NaOH-extractable inorganic and organic P contents. Soil P availability was parameterised by IEK methods.

Materials and methods

Sample collection and experimental setup using mesocosms

The collection area is located in the Malagasy Highlands under altitude tropical climate with a dry season from May to October and a humid one from November to April. Experimental designs were established on *Tanety* (hills) in 2002 and 2006, respectively, at Andranomanelatra and Lazaina sites, both on long-term grassland dominated by *Aristida* species. The experimental designs included traditional hand tillage and DMC systems with various crop rotation and fertilisation treatments. An uncleared part of the *Aristida* sp. fallows was kept at both sites to serve as reference plots. Paired samples of earthworms and surrounding soil necessary for the mesocosm study were collected in the control plots at both sites. Selected properties of these Ferralsols (FAO 1998) are presented in Table 1. Soil samples were collected in the 0- to 10-cm depth layer, air-dried and sieved through a 2-mm sieve. Adults of *Pontoscolex corethrurus* (Glossoscolecidae), an endogeic geophagous earthworm species commonly found in tropical agricultural soils, were collected during the wet season on each site and used in the experiment. The individuals were kept in the corresponding soil at 28°C until the experiment started.

Table 1 Main physico-chemical properties of the topsoil (0- to 10-cm depth) at Andranomanelatra and Lazaina

	Andranomanelatra (19°47′ S, 47°06′ E)	Lazaina (18°46′ S, 47°32′ E)
Sand, g (kg soil)$^{-1}$	190	580
Silt, g (kg soil)$^{-1}$	190	70
Clay, g (kg soil)$^{-1}$	620	340
Carbon, g (kg soil)$^{-1}$	46.9	21.6
Nitrogen, g (kg soil)$^{-1}$	3.7	1.5
pH (H_2O)	5.0	5.4
Fe_{ox}[a], g (kg soil)$^{-1}$	3.5	0.3
Al_{ox}[a], g (kg soil)$^{-1}$	12	0.9
Fe_{DCB}[b], g (kg soil)$^{-1}$	47	4.2
Al_{DCB}[b], g (kg soil)$^{-1}$	17	1.9

[a] Fe and Al as determined by atomic adsorption spectrophotometry in ammonium oxalate–oxalic acid (pH=3)
[b] Fe and Al as determined by atomic adsorption spectrophotometry in dithionite–citrate–bicarbonate extracts (Mehra and Jackson 1960)

A total of 20 mesocoms were set up which correspond to two soils in which earthworms were introduced or not (control) and five replicates per treatment. The mesocosms (2.7-L metallic boxes; ten per soil) were filled with 1.8 kg of dry soil mixed with 650 ml of demineralised water to reach a 35% moisture level. The mesocosms were then pre-incubated during 7 days before soil humidity was adjusted to 40% in all boxes and earthworms added in half of the boxes. In the earthworm treatments, two adults (on average 0.6 g per living adult) of *P. corethrurus* were added, corresponding to a density of about 120 individuals per square metre, which is in line with reported values in no-tillage systems in the experimental area (Coq et al. 2007). The soil water content was individually readjusted every 2–4 days for each mesocosm. All boxes were demounted after 35 days at 28°C. In the treatments without earthworms, control soil was directly collected for analysis. In the treatments with earthworms, the individuals were in good health and active as they gained weight, produced cocoons and casts (105 g casts per box in average for the 35-day period) in all boxes. Casts were manually separated from non-ingested soil in the fraction >2,000 μm according to their globular shape. All samples were air-dried and analysed separately (i.e. per mesocosm).

Soil and cast analyses

Total P was determined in soil and casts by perchloric acid digestion (Olsen and Sommers 1982). Sodium hydroxide extractable P was determined after shaking 1 g of soil or casts in 30 mL NaOH (0.1 M) for 16 h, centrifuged for 15 min at 5,000 rpm and filtered through Whatman paper no. 40 (Bowman and Cole 1978). As recommended by Tiessen and Moir (1993), NaOH extracts were acidified and diluted before determining inorganic P (NaOH-Pi) using the ascorbic acid–ammonium molybdate method (John 1970). Its detection limit was 25 μg P L^{-1}. Total P in NaOH extracts were also determined similarly after oxidation in the autoclave with NH_4-persulfate (Tiessen and Moir 1993). NaOH-extractable organic P (NaOH-Po) was calculated as the difference between total and Pi contents of the extracts.

Acid phosphomonoesterases (PME) activity was estimated by the para-nitrophenyl phosphate tetrahydrate method (Tabatabai 1982). The soil or cast samples were incubated for 1 h at 37°C, the reaction terminated by alkalinisation and the absorbance determined spectrophotometrically at 410 nm. Controls were processed on each sample to determine the non-enzymatic hydrolysis of the substrate and to correct for background coloration. Enzymatic activities are expressed as the concentration of the hydrolysis product [micrograms *p*-nitrophenol (NP) (per gram soil) per hour].

Isotopic exchange kinetic experiment

The Pi exchange between solid and liquid phases of suspended soils was assessed following the ^{32}Pi labelling and dilution procedure proposed by Fardeau (1996) and often used afterwards (Frossard and Sinaj 1997; Morel et al. 2000; Stroia et al. 2007). Briefly, for soil and cast samples, 1 g was suspended in 10 mL of distilled water. All suspensions were shaken on a shaking table for 16 h to reach a steady state during the subsequent isotopic dilution kinetics. Then, 50 μL of carrier-free ^{32}Pi (having a radioactivity level R of about 40 MBq) was introduced into the soil suspension at time zero. At times (t) corresponding to 4, 40 and 425 min of exchange, respectively, 2.5 mL of soil suspensions were sampled with a plastic syringe and immediately filtered through 0.2-μm membrane filters (Minisart, Sartorius). In parallel, the same procedure was carried out in distilled water only (without soil) to determine R. Both R and the radioactivity remaining in solution (r) after each time (t) were counted altogether to account for isotopic decay and eventual quenching effect using a liquid scintillation cocktail with a counter (Perkin Elmer Tri-Carb 2800 TR). The isotopic dilution ratio (r/R, dimensionless) was calculated. The Pi concentration (C_p) in filtered soil solution was determined using the malachite green colorimetric method (van Veldhoven and Mannaerts 1987) with a cell of 10-cm length path; the detection and quantification limits were 3 and 8 μg P L^{-1}, respectively.

The isotopically exchangeable Pi amount (E) was calculated applying the basic assumption that in any fraction of E, the isotopic composition (IC) of Pi measured after addition and exchange of carrier-free ^{32}Pi is identical to the isotopic composition of Pi in the soil solution (Fardeau 1993):

$$\mathrm{IC} = \frac{R}{E} = \frac{r}{Q_w} \text{ which gives } E = \frac{Q_w}{r/R} \quad (1)$$

where Q_w (mg P kg^{-1}) is the Pi amount in solution [$Q_w = C_p$ (mg P L^{-1} solution) multiplied by the volume-to-mass ratio of soil suspension which is 10]. The E value included dissolved Pi amount and gross amount of diffusive Pi. For all soil and cast replicates, a data set of three C_p and E values was obtained.

Data analyses and statistics

Where the variances were not homogeneous, a logarithmic transformation of data was applied. Differences between means per site and sample type for total P, NaOH-extractable Pi, Po and Pt, and PME activity were assessed by the software package Statistica 6.1 for Windows (©Statsoft 2004) and considered significant by least significant difference using the Tukey test at $P<0.05$.

The IEK were parameterised by the following equation proposed by Fardeau (1993, 1996):

$$E = E_{1min}t^n, \text{with } E < \text{soil mineral P content} \quad (2)$$

where $E_{1\ min}$ (mg P kg^{-1}) is the isotopically exchanged Pi after 1 min of isotopic dilution; $E_{1\ min}$ represents a homogenous pool, which contains Pi in solution plus Pi onto the solid phase with the same mobility than Pi in solution. The $E_{1\ min}$ value is immediately available to crops without chemical transformation (Fardeau 1996). The n parameter is a coefficient that accounted for the ^{32}P disappearance from the solution with time, i.e. for the slow time-dependent reactions of Pi with solid constituents of soil. Both estimates of $E_{1\ min}$ and n were obtained using nonlinear, least-squares parameter optimisation (Proc NLIN) of Statistical Analysis Software (SAS Institute 1995).

Results

P components and phosphomonoesterase activity in soil and derived casts

Total P (Pt) content was lower in Lazaina than in Andranomanelatra samples (325–406 vs. 836–906 mg P kg^{-1}, respectively), whilst no significant difference was observed between control, non-ingested soils and casts in both sample series (Table 2). Accordingly with total P, NaOH-extractable total P, the sum of inorganic (NaOH–Pi) and organic (NaOH–Po) P was lower in Lazaina (38–47 mg P kg^{-1}) than in Andranomanelatra samples (178–186 mg P kg^{-1}). Both NaOH-Pi and NaOH-Po represented 11% and 22% of the total Pt values for Andranomanelatra and Lazaina soils, respectively. NaOH-Po content, representing more than 70% of the NaOH-extractable total P, was significantly increased by earthworms, whilst no difference was observed between control soil and casts in NaOH-Pi (Table 2). The increase in NaOH-Po between soil and casts was 5 mg kg^{-1} for Andranomanelatra and 9 mg kg^{-1} for Lazaina. Phosphomonoesterase activity ranged from 166 to 180 μg nitrophenol per gram per hour and from 240 to 315 μg nitrophenol per gram per hour in Lazaina and Andranomanelatra samples, respectively (Table 2). In both cases, phosphomonoesterase activity was lower in casts than in the control or non-ingested soils, although the difference was not statistically significant.

Pi concentration in soil solution (C_p) and isotopically exchangeable Pi kinetics (IEK)

The Pi concentration in soil solution (C_p) was extremely low with values around 2–3 μg P L^{-1} for Andranomanelatra and 7–10 μg P L^{-1} for Lazaina (Table 3). No statistical differences were observed for C_p values between control soils and casts in both cases. For Andranomanelatra, the C_p values were close to the detection limits of the colorimetric method and should be cautiously considered as more indicative than descriptive.

The amounts of isotopically exchangeable Pi (E) varied with elapsed time of isotopic dilution for all soils and casts (Fig. 1). For a given soil and cast, time dependence of E values is closely fitted to Eq. 2. The parameters estimates of Eq. 2, $E_{1\ min}$ and n, are presented in Table 3. No statistical difference was observed between control and non-ingested soils. By contrast, the $E_{1\ min}$ value was significantly higher in casts than in the soil from which they were derived (1.35 vs. 0.49 mg P kg^{-1} for Andranomanelatra and 3.32 vs. 2.11 mg P kg^{-1} for Lazaina, respectively). The relative increase in casts of $E_{1\ min}$ ((casts − control)/control), expressed in percentage, is +116% in average for the two soils.

The n parameters varied from 0.41 to 0.50 with values slightly lower in casts than in control soil (Table 3).

Table 2 Total P (P_t) and inorganic, organic and total NaOH-extractable P contents (mg P kg^{-1}) and phosphomonoesterase activity (PME, μg NP g^{-1} h^{-1}) in soil and casts samples derived from mesocosms filled with Andranomanelatra and Lazaina soils

Soil origin	Sample type	P_t	NaOH-extractable P			PME (μg NP g^{-1} h^{-1})
			Inorganic	Organic	Total	
		mg P kg^{-1} soil				
Andranomanelatra	Control soil	836 a	49 a	132 a	181 a	311 a
	Non-ingested soil	870 a	50 a	128 ab	178 a	315 a
	Casts	906 a	52 a	134 c	186 b	240 a
Lazaina	Control soil	349 a	8 a	30 a	38 a	180 a
	Non-ingested soil	343 a	6 a	33 a	39 a	166 a
	Casts	406 a	8 a	39 b	47 b	169 a

In a column, different letters indicate significant difference between samples within a site ($P<0.05$)

Table 3 Concentrations of P ions in solution (C_p) and $E_{1\ min}$ and n parameters describing isotopic exchange kinetic in samples derived from mesocosms filled with Andranomanelatra and Lazaina soils

Soil origin	Sample type	Cp (μg P L^{-1})	$E_{1\ min}$ (mg P kg^{-1})	n	Obs.	R^2
Andranomanelatra	Control soil	2 (2)	0.49 (0.19)	0.50 (0.07)	15	0.92*
	Non-ingested soil	2 (2)	0.52 (0.14)	0.44 (0.05)	15	0.94*
	Casts	3 (2)	1.35 (0.20)	0.41 (0.03)	9	0.99*
Lazaina	Control soil	7 (2)	2.11 (0.43)	0.45 (0.03)	15	0.97*
	Non-ingested soil	7 (2)	1.94 (0.47)	0.47 (0.04)	15	0.96*
	Casts	10 (2)	3.32 (0.24)	0.41 (0.01)	9	>0.99*

Means with standard deviation in parentheses. *Obs*. denoted the number of experimental values: three times × five replicates for control and non-ingested soils; three times × three replicates for casts. R^2 is the proportion of variation accounted for by Eq. 2
*$P<0.0001$; probability level for Eq. 2 $E=E_{1\ min}\ t^n$

Discussion

The total P (P_t) contents observed in the present study are within the expected range for Ferralsols. In a recent review on P in tropical soils, Nziguheba and Bünneman (2005) found that P_t ranged from 61 to 1,780 mg P kg^{-1}. In our experiment, only a small proportion of Pt (11–22%) was extracted by NaOH reagent, whilst in sequential extractions of P from Ferralsol, Friesen et al. (1997) extracted 40–50% and Tiessen and Moir (1993) about 60% of total P in NaOH-extractable fractions of coarser textured soils. In our study, for both soils, Pi concentrations in solution (C_p) were extremely low (<20 μg P L^{-1}), whilst the total P contents were relatively high. For such C_p values, P is a limiting factor for crop production (Sinaj et al. 2001; Rabeharisoa 2004). In tropical soils, limited P availability to plants can coexist with large total P contents (Nziguheba and Bünneman 2005). The $E_{1\ min}$ values, which assessed the immediate ability of soil to supply Pi without chemical transformation, are therefore a relevant indicator of the P availability to plants (Fardeau 1993). The $E_{1\ min}$ value ranged from <1 to >100 mg P kg^{-1} depending on soils, field P fertilisations and P additions in laboratory batch experiments (Fardeau et al. 1991; Morel et al. 1994, 2000; Frossard et al. 1995). When $E_{1\ min}$ value is lower than 5 mg P kg^{-1} as observed in the present situations, the soil should be considered as very poor in available soil P (Fardeau et al. 1996). The lower C_p and $E_{1\ min}$ values in the Andranomanelatra soil can be attributed to its higher concentrations of clay and Fe oxides (62% clays; 3.5 g oxalate-Fe kg^{-1}; 47 g dithionite-Fe kg^{-1}) compared with the Lazaina soil (34% clays; 0.3 oxalate-Fe kg^{-1}; 4.2 g dithionite-Fe kg^{-1}; Table 1). Several studies have reported strong positive correlation between high buffering capacity and iron and aluminium oxide contents in soils (Frossard et al. 1995; Hooda et al. 2001).

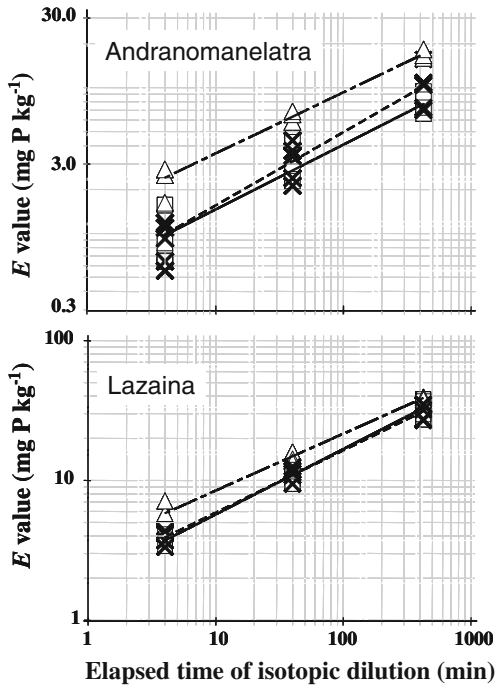

Fig. 1 Kinetics of isotopically exchangeable P ions (E, mg P kg^{-1}) transferred between solid and liquid phases and associated regression line (E vs. t) in control (*ex*; *dashed lines*), non-ingested (*square*; *solid lines*) soils and casts (*triangle*; *semi-dashed lines*) of Andranomanelatra and Lazaina sample series. *X* and *Y*-axes are represented in log–log scales

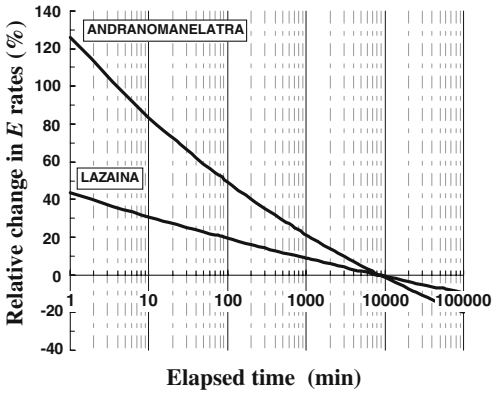

Fig. 2 Kinetics of the relative (casts − control)/control) change in E rates, expressed in %, for both soils. *X*-axis is represented in log scale

The mesocosm experiment provides a better understanding of the dynamics of P in soil and in earthworm casts. The density of two earthworms per box used in the experiment corresponded to about 120 individuals per square metre. Such a density was observed in no-tillage systems of the experimental design at Andranomanelatra (Coq et al. 2007). If casts were produced at a similar rate in field during the wet season (5 months per year), the observed production in mesocosm (105.3 g per box in average for the 35-day period) would roughly correspond to 250 t of soil ingested by earthworms per year and hectare. The ingestion of soil by *P. corethrurus* significantly increased its concentrations in organic P extractable by NaOH as well as the amounts of P exchanged at the soil/solution interface within 1 min ($E_{1\ min}$), the total P content being unchanged. Chapuis-Lardy et al. (1998) also observed no change in the total P content between control, non-ingested soil and surface casts for *P. corethrurus* fed with a Peruvian Ferralsol, whilst Pi availability was increased by soil ingestion. Our results confirm the ones of Guggenberger et al. (1996) who found higher levels of NaOH-extractable organic phosphorus in earthworm casts and a marked effect on P availability if compared with soil. NaOH-extractable organic P mainly includes phosphate mono and diesters. PME form an important group of enzymes catalysing the hydrolysis of phosphomonoesters to orthophosphate. The absence of difference between control soil and casts in NaOH-extractable inorganic P may be linked to the absence of difference in enzymatic activity. The higher P availability ($E_{1\ min}$ value) in casts may be linked to a rapid turnover of the increased organic P content. This assumption could not be verified without labelling of the soil with radioactive P before chemical extraction as performed by others (Bünemann et al. 2004). López-Hernández et al. (1993) also reported that higher P availability in fresh casts of *P. corethrurus* and ascribed the increase to changes in sorption complexes induced by competition for sorbing sites between orthophosphates and carboxyl groups of a mucus glycoprotein produced in gut. Greater phosphate availability in *P. corethrurus* casts can also be attributed to a greater pH of the gut content (Barois and Lavelle 1986) along the earthworm intestinal tract (6.0–6.8) compared to the soil (5.0–5.4) that affects P sorption in casts.

The *n* parameter of IEK ranged from 0 to 0.5 (Fardeau 1993). It indicates the contribution of slow reactions to replenish Pi into solution. The higher is the *n* parameter, the higher is the relative importance of slow reactions. If *n* value is 0, the time effect on IEK is nil. But if *n* value is 0.5 as for Andranomanelatra control soil, the *E* value increased by $10^{0.5}=3.2$ when elapsed time increased by 10. Thus, assuming that Eq. 2 is valid for 1 week (almost 10,000 min), the $E_{10,000\ min}$ values are $10{,}000^{0.5}=100$ times higher than the $E_{1\ min}$ value. For the casts collected in Andranomanelatra, this factor became $10{,}000^{0.41}=44$. Thus, the time effect assessed over this period is less marked in casts than in control soil. The same calculation for Lazaina soil gave 63 ($10{,}000^{0.45}$) and 44 ($10{,}000^{0.41}$) for control and casts, respectively. In average for the two soils, the relative effect of time on *E* value assessed by this factor is a decrease by −44%. To analyse the consequence of the decrease of *n* parameter in casts, we calculate the *E* rate, i.e. the first derivative to time of *E* values, for control soils and casts. Then, we calculate the relative ((casts − control)/control) change of *E* rates, expressed as percentage (Fig. 2). For both soils, the pattern is similar. Relative *E* rate is the highest initially and then subsequently decreased with time. Then, after 10,000 min, the *E* rates became negative. The overall effect of earthworm ingestion on IEK corresponds to transformations of slowly exchangeable Pi towards quicker exchangeable Pi. Barois et al. (1993) showed that the soil structure was completely destroyed during the gut transit in *P. corethrurus* and that the soil was fully reorganized, producing newly formed aggregates and specific microstructure in casts. Such changes along with a selective ingestion of small particles (Chapuis-Lardy et al. 1998) may have contributed to the shift between exchangeable phosphate pools.

Conclusion

The very low P concentration in soil solution and $E_{1\ min}$ value, considered immediately and totally available to plants, emphasise the deficiency in immediate available Pi even after soil ingestion by earthworm. However, the earthworm casting activity improved soil P availability through an increase of the exchangeable P within 1 min ($E_{1\ min}$). Earthworm ingestion induced transformations of slowly exchangeable Pi towards quicker exchangeable Pi. Additional experiment would also be useful to determine if such an increase in P availability will be sufficient to affect crop nutrition and growth, as the C_p and $E_{1\ min}$ values in casts remained below the critical values of 20 μg L^{-1} and 5 mg P kg^{-1}, respectively. Probably, the presence of earthworms alone (even if numerous and active) will not eliminate the P deficiencies. Therefore, such soils will require inputs of mineral P fertiliser for plant growth and increased crop production. Further research should focus on the efficiency of the P fertilisers in the Malagasy agricultural systems when combined with the presence of earthworms.

Whilst mesocosms experiments remained crucial to understand process at small-scale levels, coupled modelling approaches can be useful to fully understand the complex interplay of biogeochemical interactions and scale-up from cast levels to soil profile and entire ecosystem (Standing

et al. 2007; Blanchart et al. 2008). This issue is becoming the primary challenge of soil functional ecology.

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