Physicochemical aspects of recycling tree leaf litter in the south of Western Siberia by the *Eisenia fetida* (Savigny) vermiculture

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Abstract. The utility of the compost worm *Eisenia fetida* (Savigny) for recycling mixed leaf litter of the tree species characteristic of the forests in the south of Western Siberia and used in the landscaping in the city of Tomsk has been demonstrated. The tree species that are the major contributors to the leaf litter in the examined area include the genera *Populus*, *Salix,* and *Betula.* Two-fraction substrates for leaf litter vermicomposting and conventional composting (decomposition with and without earthworms) were prepared of the harvested and dried leaf litter. The feeding fraction consisted of leaf litter moistened with distilled water and the absorbing fraction, of alluvial river sand. The physicochemical properties of the studied leaf litter were weakly acidic pH of aqueous extracts, a very low content of nitrate nitrogen, and a relatively low K^+ concentration. The prevalent cation in the assayed leaf litter was Ca^{2+}. The leaf litter was partially decomposed on the surface of sand substrates during 35-day incubation under humid conditions; accumulation of inorganic ions in the sand was one of the signs indicating this decomposition. Ca^{2+} was also prevalent among these ions.

1. Introduction

Degradation of the soil cover and reduction in the area of farming lands are among the most serious agroecological challenges. In this situation, intensification of agriculture and quality improvement of the produced crops are of special importance. The decrease in the yields of crops associated with an increase in the abundance of pests and diseases is also a relevant problem. This entails the need in soil application of chemicals for plant protection, which has a negative impact on the product quality and state of the soils. This has brought to the fore the necessity to produce ecologically friendly fertilizers, which, on the one hand, would stimulate plant growth and, on the other, would increase their nonspecific resistance to pests, diseases, and stresses. Vermicompost, the product resulting from conversion of organic substance by earthworms, is this particular kind of fertilizers [1, 2].

The earthworms are currently used for production of organic fertilizers and utilization of the waste of human economic activities on a global scale [3, 4]. A tremendous amount of work has been done during the last decades on selecting the worm species and hybrids best fitting the vermicomposting technology. One of these species is the redworm *Eisenia fetida* (Savigny). This is a ubiquitous species readily adaptable to manifold organic substrates [5]. According to ecological classification, *E. fetida* worms belong to the so-called epigeic category [6]. In wildlife, the epigeic worms inhabit the upper soil layer and the ground litter on the surface [7]. An adequate habitat for epigeic worms is easily created under laboratory conditions. The species *E. fetida,* a source of laboratory and technological cultures, significantly exceeds many other earthworm species in its fecundity, is well culturable under artificial conditions, and is most frequently used for vermicomposting organic waste [8]. A general worldwide trend in vermicultivation is that the *E. fetida* worms recycle the animal and poultry farm manure. However, the issue of vermicomposting, which implies recycling nitrogen-poor substrates with high cellulose content, is still insufficiently considered in both agricultural practice and research, though the ideas on how to solve technological problems of this type with the help of vermiculture appeared rather long ago. One of such problems is recycling leaf litter, which comprises several important aspects [9, 10]. First, burning of leaf litter, frequently practiced now, is a rather ecologically unfriendly measure; moreover, leaf litter natural decomposition is a necessary component of biogeochemical cycles and trophic chains. Second, the leaf litter contains certain mineral elements in a hardly accessible (poorly soluble) form. Composting considerably enhances extraction of these substances and their intake by plants. This makes it possible to use the processed leaf litter as a fertilizer. Calcium is a component of special concern among the mineral nutrient elements from the standpoint of its extraction from leaf litter and return to the root horizons [11, 12]. During the plant life activities, calcium concentrates in the older leaves as oxalates and other almost insoluble compounds. In several cases, it is almost impossible to return this element to the root horizons (in the initial amounts). Earthworms allow for solving this problem: they possess unique specific anatomical and physiological features associated with extraction of calcium from the substrate and its further metabolism [13], namely, the so-called calciferous glands [14]. The worms convert calcium salts into coprolites by passing the organic waste and leaf litter through their digestive tract [15]. Although the leaf litter is very poor in nitrogen and relatively poor in potassium, the presence of manifold calcium salts makes it a promising (and readily accessible) substrate for producing the vermicompost enriched for calcium compounds. This makes it possible to use the vermiculture product as an organomineral fertilizer with a positive effect on root formation and nonspecific resistance of a plant organism. The role of calcium in an increase in the nonspecific plant resistance is well known [16-18].

The **goal** of this work was to study the main physicochemical aspects in the vermicomposting of tree leaf litter by the *E. fetida* culture.

2. Materials and methods

In the experiments, alluvial river sand from shorelines of small Western Siberian rivers with low-mineralized water and prevalent water supply from high bogs was used as an absorbing material. The pH and conductivity of the peat aqueous extracts were monitored by potentiometric and conductometric methods, described in [19, 20]. The pH and electric conductivity of the sand aqueous extracts (1 : 10) were 4.5 and 28 $\mu S \times cm^{-1}$, respectively. This conductivity value corresponds to the total content of electrolytes not exceeding 2 mEq/kg sand dry weight (DW). Low pH values promoted the initial phase in decomposition of poorly soluble salts contained in the leaf litter. In addition, the use of sand, almost free from electrolytes (at the level of macroelements), allowed us to study some physicochemical processes of the leaf litter decomposition (passing of minerals from the leaf litter fraction into sand fraction) in a pure form without any additional interfering factors, such as inorganic ions.

As the food component of the substrates to be vermicomposted, we used a mixed leaf litter of the trees growing in the University Grove (*Universitetskaya Roshcha*), the natural complex which is a part of the historical and architectural ensemble of Tomsk State University (56°28′08″ N, 84°56′55″ E). The following tree species provide the major part of the tree leaf litter in this habitat: the genera *Populus* (including *P. nigra* L., *P. balsamifera* L., and hybrid species), *Salix* (including *S. alba* L. and *S. fragilis L.*), and *Betula* (including *B. pendula* Roth.). These species are abundant in the local dendroflora and are used in landscaping of Siberian cities, Tomsk included.

The experiments were performed in 750-mL containers each filled with 400 g of dried washed river sand, moistened with distilled water at the start of experiment. The humidity of the substrates during vermicomposting was maintained at 75 ± 10% by adding distilled water on a regular basis. The need in correcting humidity was assessed gravimetrically. Then three 70-mL glass cylinders were placed in each container; each cylinder was filled with leaf litter, which was also moistened with distilled water. The cylinders were placed horizontally on the sand surface with their top open to allow the worms to enter the cylinders and leave them for sand substrate. Thus, the two-fraction substrates comprising the feeding and absorbing components were prepared. The control containers were supplemented only with the initial feeding substrate but not worms. The *E. fetida* worms were placed in the experimental containers at a total weight of 5 ± 0.5 g/container. The containers closed with perforated lids over the experiment (35 days) were kept in a dark room at an air temperature of +21 ± 3°C. Thus, the leaf litter substrate was composted without earthworms in the control containers and vermicomposted in the experimental ones. On completion of the experiments, the leaf litter and sand substrates were weighed and dried in a drying oven at t = 105°C.

The extracts of experimental substrates were made of dry weighed samples of sand (10 g) and minced leaf litter (1 g). Distilled water was added to the weighed samples at a ratio of 1 : 9 for sand and 1 : 99 for leaf litter and quantitatively transferred to dark glass bottles, capped, stirred for 3 min in a shaker, and left at a room temperature (+21 ± 3°C) for 24 h. The extracts were then filtered and assayed to measure electric conductivity, pH, Ca^{2+} content [21] (by complexometry), and K^+ and NO_3^- concentrations [22] (by ionometry).

All experiments were performed in nine replicates. The Shapiro–Wilk test for normality of all studied samples complied with the application of parametric statistical methods. The data are presented as mean arithmetic values and their errors. Student's t-test for independent samples was used to compare two means.

3. Results and discussion

Table 1 lists the measured physicochemical parameters for the extracts of mixed leaf litter used in the experiments.

Table 1. Physicochemical parameters of forest leaf litter from the University Grove of Tomsk State University.

Parameter	Value
pH of water extract	6.08 ± 0.09
EC of water extract, [$\mu S \times cm^{-1}$]	651.89 ± 65.87
K^+, [mEq/kg DW]	259.96 ± 18.98
NO_3^-, [mEq/kg DW]	23.48 ± 2.03
Ca^{2+}, [mEq/kg DW]	464.38 ± 92.77*

Note: mEq, milliequivalent; DW, dry weight; EC, electric conductivity; *significant difference between calcium and potassium concentrations, $p < 0.05$.

As is evident from Table 1, the leaf litter extracts were weakly acidic. The conductivity was sufficiently high, corresponding to the total electrolyte content of approximately 500 mEq/kg DW. The concentration of nitrate was very low, which matches the relevant published and our own data that the leaf litter is a nitrogen-poor substrate as compared with the content of carbon and cationic macroelements [23-25].

The K^+ concentration in leaf litter samples on the average amounted to 260 mEq/kg DW. Two main approaches to assess the concentration of this macroelement in plant tissues are used: agrochemical, when the concentration is expressed in percent or grams per kilogram DW, and physiological, when the concentration is expressed in terms of milliequivalents per kilogram of wet weight (mEq/kg WW). The K^+ concentration in the studied leaf litter (260 mEq/kg or 10.14 g/kg DW) in general agrees with the published data on the accumulation of this macroelement in tree leaves (including evergreen species). According to Wang and Moore [26], this parameter varies in green leaves in the range of 2.5–25 g/kg K^+ depending on the season and plant ecotype. On the other hand, in terms of physiology, 260 mEq/kg DW corresponds to approximately 55–65 mEq/kg WW, which certainly cannot be regarded as a normal physiological level for potassium content in living plant tissues, normally confined to the range of 100–200 mEq/kg WW [27].

The Ca^{2+} concentration in the leaf litter was high and exceeded twofold the K^+ concentration. The values of conductivity and the above data on the content of potassium and nitrate in leaf litter demonstrate that the prevalent macroelement in the studied mixed leaf litter is calcium. This agrees well with the patterns of ontogenetic changes in the mineral nutrition status of the higher glycophytes. These patterns have been comprehensively studied [28, 29]. One of the general trends is that the leaves (both of trees and herbaceous plants) during ontogenesis gradually lose potassium (owing to its constant outflow to younger organs and subsequent reutilization) but increasingly accumulate calcium. This macroelement in aging and dying leaves is fixed in a form of oxalates and other poorly soluble compounds; however, a certain amount of calcium ions along with potassium and nitrate ions is still present in the tissues of leaf litter. As we have earlier demonstrated, the content of water-soluble calcium in the leaves of some plant species can also reach more than 150 mEq/kg WW [30].

The main quantitative characteristic of the degree of leaf litter decomposition in our experiments is the accumulation of ions in the sand fraction of vermicultivation substrate.

Table 2 presents the data of analytical measurements of the sand fraction extracts after 35-day exposure of leaf litter in vermiculture and in control containers (without earthworms).

Table 2. The physicochemical parameters of sand fraction after composting and vermicomposting of the forest leaf litter from the University Grove of Tomsk State University.

Parameter	Variant	
	Control	Vermiculture
pH of water extract	4.62 ± 0.9	5.33 ± 0.06***
EC of water extract, [$\mu S \times cm^{-1}$]	80.91 ± 3.05	116.67 ± 3.27***
NO_3^-, [mEq/kg DW]	Below detection threshold	Below detection threshold
K^+, [mEq/kg DW]	0.44 ± 0.09	1.62 ± 0.16***
Ca^{2+}, [mEq/kg DW]	3.56 ± 0.17	5.27 ± 0.08***

Note: *** Significant difference with control at $p < 0.001$.

The pH of the sand extracts from the control containers on completion of the experiment remained almost at the same level (for the used sand), approximately 4.6. In both the control and experiment, the concentration of nitrate ion was very low, preventing from measuring it by ionometry. However, the other parameters suggested decomposition of leaf litter. In particular, the pH of the sand fraction from experimental containers increased as compared with the control (and the initial values for sand). The conductivity of aqueous sand extracts of moistened leaf litter after the incubation increased approximately threefold as compared with the initial values for the control and more than fourfold, for the experiment. The K^+ concentration in the control sand samples was very low versus the experiment, where this concentration reached 1.6 mEq/kg. Certainly, the prevalent ion in the extracts of the sand fraction of both the control and experiment was Ca^{2+}. As is evident from Table 2, pH, conductivity, and concentrations of K^{+} and Ca^{2+} in the extracts of the experimental substrates were higher in a statistically significant manner as compared with the corresponding control samples.

In our view, the data on the change in pH in the vermicomposted substrates in combination with the data on electric conductivity and accumulation of cations highlight the main aspects of the leaf litter vermicomposting: the earthworms recycle the leaf litter by passing it through their digestive tract and convert a considerable portion of the deposited elements of plant mineral nutrition into a better soluble form (as compared with the initial leaf litter) [31]. Of these macroelements, calcium is absolutely quantitatively prevalent. This agrees well with the data by Versteegh et al. [32] and Reich et al. [12] that one of the most important physicochemical aspects of the tree leaf litter recycling by earthworms consists in the transfer of calcium from leaf litter to soil and neutralization of excess soil acidity. Note also that the calcium in leaf litter is mainly contained as an oxalate [29], being almost insoluble and, consequently, inaccessible for plants and the processes neutralizing the excess soil acidity. The calciferous glands of earthworms sorb calcium and convert it into carbonate [13, 14, 33], which is almost similarly insoluble as oxalate; however, carbonate is convertible into bicarbonate under certain conditions (for example, low pH values), which is the main source of Ca^{2+} in the soil solution. The leaf litter is decomposed in the absence of worms as well owing to the activities of saprophytic fungal communities; however, earthworms considerably accelerate this process. This is confirmed by both our experiments (Table 2) and published data [11, 34, 35].

4. Conclusion

Our study has demonstrated that the *E. fetida* vermiculture can be efficiently used for recycling the leaf of the tree species characteristic of the Western Siberian dendroflora and used in city landscaping. Although the degree of calcium gland development in the earthworm *E. fetida* is relatively low (as compared with the soil worm species), a high technological efficiency (fecundity, ecological plasticity, and ease of keeping) of this compost species allows for its successful use for recycling leaf litter. Further studies in this direction may expand the potential of vermicomposting application aiming to increase the functional efficiency of urban and agricultural ecosystems [36].

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