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# Vermicomposting as a Sustainable Option for Managing Biomass of the Invasive Tree *Acacia dealbata* Link

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**Abstract:** The tree *Acacia dealbata* is native to Australia but has become invasive in many parts of the world thanks to its N-fixing capacity and to the allelopathic compounds present in its biomass. We conducted a pilot-scale study to assess the potential conversion of *A. dealbata* biomass by vermicomposting via the earthworm *Eisenia andrei*. The flowering aerial *A. dealbata* biomass was shredded and placed in a vermireactor under greenhouse conditions for 56 days. The vermicomposted material was sampled every two weeks to analyse its biological and chemical parameters. The phytotoxicity of the fresh *A. dealbata* material and vermicompost was assessed via an ecotoxicological test with *Lepidium sativum* seeds. The activity of the earthworms caused strong modifications of the properties of the processed material: the electric conductivity, basal respiration, and organic matter content were reduced, whereas the concentrations of other elements such as N, P, or Zn increased. The earthworm biomass increased steadily until day 42 and then decreased, probably due to the depletion of labile organic matter during the initial stages of vermicomposting. The fresh *A. dealbata* material reduced the germination and radicle elongation of *L. sativum*, whereas vermicompost showed the same values as control. The produced vermicompost was an organic fertiliser rich in N and was not phytotoxic. Vermicomposting provides an opportunity to create a new value chain for the control of the invasive tree *A. dealbata*.

**Keywords:** *Eisenia andrei*; epigeic earthworms; germination tests; organic fertiliser; phytotoxicity

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## 1. Introduction

*Acacia dealbata* Link (Fabaceae) is native to Victoria, New South Wales, and Tasmania (Australia), where it can grow as a shrub or tree, reaching heights of up to 30 m [1]. *A. dealbata* is a fast-growing species with showy, scented flowers. It is able to develop in nutrient-poor soils thank to its ability to fix atmospheric nitrogen [2]. During the nineteenth century, *A. dealbata* was introduced in Europe, India, South Africa, and South and North America for multiple purposes: ornamental use, wood and tannins production, dune stabilization, or for the revegetation of bare land [3–7]. After its introduction in new areas, *A. dealbata* has expanded its range and is currently considered an invasive weed in SW Europe (Spain, Portugal, France, and Italy), South Africa (Cape floristic region), and areas with a Mediterranean climate in Chile [3,8]. The invasive behaviour of *A. dealbata* has been explained by the presence of several traits: a tolerance for changing soil conditions (including a capacity for nitrogen fixation and high water consumption), the ability to take advantage of environmental disturbance, phenotypic plasticity, vegetative reproduction (by the presence of rhizomes), fire tolerance (with the capacity to resprout and germinate profusely after forest fires), and an allelopathic potential [5,6,9]. Several compounds with phytotoxic effect have been found in *A. dealbata*, such as alkaloids, cyanogenic glycosides, terpenes, flavonoids, and tannins [7]. Although these compounds are mainly present during the flowering period [10], some studies have indicated a phytotoxic effect throughout the entire phenological cycle [7,11]. In spite of this

recognised phytotoxicity of *A. dealbata's* biomass, recent studies indicate that allelopathy may be a secondary factor in the success of this species, whereas modifications in microhabitat induced by *A. dealbata* after its colonisation of a site constitute the main driver affecting seedling establishment in the European non-native range [12].

In Galicia (NW Spain), *A. dealbata* has extensively invaded natural forests, abandoned arable land, and water courses, causing an important impact on the native flora and even impacting protected areas [5,9]. According to these impacts, this species has been included since 2013 in the Spanish Catalog of Invasive Alien Species [13]. The inclusion of a species in this Catalog entails, among other measures, a generic prohibition of the possession, transport, traffic, and trade of live specimens, their remains, or propagules that could survive or reproduce, as well as the preparation of a control and eradication program for those species considered priority risks [14].

Although some biological control measures have been proposed for *A. dealbata* and other *Acacia* species in Europe [3,5] and tested in South Africa [15], the main strategy used is the mechanical removal of the aerial parts, sometimes in combination with the use of herbicides to reduce resprouting [13,16]. However, this process is expensive and generates biomasses with potentially negative effects on the flora and soils due to the presence of allelopathic compounds.

Recently, some authors have proposed the creation of value chains from *A. dealbata*, either by its use as biofuel or as a source of valuable compounds and biochemicals, as a way to improve the control and eradication of this invasive species [17,18]. Here, we propose an alternative: the conversion of *A. dealbata* biomass into a fertiliser by vermicomposting.

Vermicomposting is the managed processing of organic waste by the synergistic action of earthworms and microbial communities [6]. The enzymes involved in the biochemical decomposition of organic matter are produced by microorganisms, and earthworms accelerate this decomposition by comminution and mixing of plant debris, which boosts the activity of bacteria and saprophytic fungi. Vermicomposting is based on the use of epigeic earthworms, which are litter dwellers that live in organic horizons [19]. In spite of the high diversity of terrestrial earthworms, with around 4000 species described to date [20], only four species are routinely used in vermicomposting facilities: *Eisenia andrei* (Bouché), *E. fetida* (Savigny), *Perionyx excavatus* (Perrier), and *Eudrilus eugeniae* (Kinberg) [21]. Previous studies have demonstrated the feasibility of vermicomposting for converting organic waste, such as sewage sludge, paper waste, urban residues, and animal waste (see [19,22] for reviews), into organic fertilisers. Vermicomposting has also been shown to be effective for processing plant wastes rich in phytotoxic compounds, such as grape marc, spent coffee grounds, and Scotch broom biomass [23–25].

In this research, we explored the potential of vermicomposting as a sustainable option for the management of *A. dealbata* biomass and its conversion into an organic fertiliser. We had two objectives: (i) to estimate whether the earthworm *E. andrei* was able to degrade the biomass of *A. dealbata*, rich in secondary compounds, and (ii) to assess the potential phytotoxic risk of the obtained vermicompost. For this purpose, we conducted a pilot-scale vermicomposting trial and combined the analysis of biochemical parameters throughout the process with an ecotoxicological bioassay of the phytotoxicity of the final product.

## 2. Materials and Methods

### *2.1. Plant Material*

Several young (around 5 m in height) silver wattle trees (*Acacia dealbata* Link) were cut down in a forest near the University of Vigo (NW Spain) in spring, when the trees were flowering. Young branches, leaves, and flowers were manually collected from those fallen trees. The branches were chopped into smaller pieces of 3–6 cm, whereas the flowers and leaves were left intact. The fresh weight of the total sample was 120 kg.

### *2.2. Vermicomposting Setup*

The collected material was processed in a rectangular, metal, pilot-scale vermireactor (length 4 m × width 1.5 m wide × height 1 m) held in a greenhouse without temperature control. Before the material was added to the vermireactor, it contained a layer of vermicompost (12 cm height), which acted as a bed for the earthworms (*Eisenia andrei*). The initial earthworm density in the vermireactor was 344 ± 10 individuals per $m^{-2}$, with a mean biomass of 99.4 ± 4.5 g $m^{-2}$. The vermicompost layer was covered by a plastic mesh (5 mm mesh size), and the tree material was added to the vermireactor in a layer (12 cm) on top of the plastic mesh. The plastic mesh allowed earthworms to move between the layers but prevented mixing of the processed material and the vermicompost bedding. It also simplified sampling of the processed material during vermicomposting.

### *2.3. Data Collection*

The density and biomass of the earthworm population was determined periodically by collecting 10 samples (five from above and five from below the plastic mesh) of the material in the vermireactor with a core sampler (7.5 cm diameter and 12 cm height) at each sampling time. Sampling was conducted every 14 days during the vermicomposting trial and ended after 56 days. In total, 50 samples were collected. At the end of the experiment, a total of 21 kg (fresh weight) of vermicompost had been produced.

In order to estimate the evolution of the physico-chemical properties of the substrate along the vermicomposting process, we divided the surface of the vermireactor into five sections and removed two subsamples (10 g) at random from each section at the beginning of the experiment (day 0) and after 14, 28, 42, and 56 days of vermicomposting. The two subsamples from each section were bulked and stored in plastic bags at −80 °C until analysis. In total, 25 samples of substrate were collected and analysed.

### *2.4. Physico-Chemical Analyses*

The moisture content was assessed after drying the samples for 24 h at 105 °C. The organic matter (OM) content was determined from the weight loss after combustion in a Carbolite CWF 1000 muffle furnace at 550 °C for 5 h. The pH and the electrical conductivity (EC) were measured in aqueous extracts (1:10 *w*/*v*) with a Crison MicropH 2000 pH meter and a Crison CM35 conductivity meter, respectively. Samples were oven-dried at 60 °C. Total C and N content were determined in dry samples in a Carlo Erba EA 1108 CHNS-O 1500 C/N analyser. The total P, K, Ca, Mg, S, Fe, Mn, B, and Mo concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) in dry, ground samples that were subjected to microwave-assisted acid digestion. The cellulose, hemicellulose, and lignin content were assessed by detergent fibre methods [26] using the FibreBag System (Gerhardt, Königswinter, Germany).

### *2.5. Microbiological Analyses*

Basal respiration was estimated from $CO_2$ production. Subsamples of the plant material and vermicompost (5 g fresh weight) were placed in 100 mL glass vessels, which were then hermetically sealed and incubated at 22 °C for 6 h. The $CO_2$ produced from the sample was trapped in 0.02 M NaOH. $CO_2$ production was quantified by titration with 0.01 M HCl to a phenolphthalein endpoint, after addition of excess $BaCl_2$. Microbial biomass carbon ($C_{mic}$) and microbial biomass nitrogen ($N_{mic}$) were determined by the fumigation–extraction method [27,28] with moist subsamples (5 g fresh weight).

### *2.6. Phytotoxicity Assay*

A germination and radicle elongation test was performed with *Lepidium sativum* L. to estimate the potential phytotoxicity of *A. dealbata* biomass (collected at t = 0) and the vermicompost (collected at the end of the experiment; t = 56). Briefly, 20 *L. sativum* seeds

were placed on a filter paper in a sterile 9 mm diameter Petri dish into which 5 mL of one of the following solutions had already been added: distilled water (control, C), 5% *w*/*v* of *A. dealbata* fresh biomass (AD5), 10% *w*/*v* of *A. dealbata* fresh biomass (AD10), 5% *w*/*v* of vermicompost (VC5), or 10% *w*/*v* of vermicompost (VC10). To prepare the AD5, AD10, VC5, and VC10 solutions, the relevant material was suspended in water and filtered after shaking for 1 h. Each treatment was prepared in triplicate, yielding a total of 15 Petri dishes and 300 seeds. After the seeds were sown, the Petri dishes were sealed with parafilm to prevent desiccation. The plates were incubated for 7 days at 20 °C in dark conditions. The plates were then collected and the number of germinated seeds in each plate was recorded. The radicle length of all germinated seeds was also recorded.

### *2.7. Statistical Analyses*

The effect of time on the earthworm density and biomass in the vermireactor was estimated by a one-way ANOVA, followed by post hoc Tukey tests. Vermicompost chemical and microbiological parameters (except the content of fibers) were analysed by Principal Component Analysis (PCA) and a reduced number of Principal Components (PCs) that captured most of the variability in the data were extracted. The PCA was based on a correlation matrix, which is appropriate for data with different measurement scales. Scores of vermicompost samples on PCs with eigenvalues higher than 1 were retained and correlated with earthworm density and biomass by the Pearson correlation index. The phytotoxic effect of *A. dealbata* biomass and vermicompost on germination was assessed by a one-way ANOVA, and the differences in radicle length were assessed by a nested ANOVA, with the replicate nested within the treatment. In case of significant differences, Tukey post hoc tests were used to determine where the differences lay. Where necessary, the data were transformed to meet ANOVA requirements. ANOVAs, PCA, and linear correlations were computed with SPSS (v. 15.0, SPSS Inc., Chicago, IL, USA).

## 3. Results

### *3.1. Changes in the Properties of A. dealbata Vermicompost during Vermicomposting*

The vermicompost produced by processing the *A. dealbata* biomass in the vermireactor for 56 days was slightly acidic (pH = 6.42), with total concentrations of N, P, and K of 39.2, 1.4, and 1.4 g $kg^{-1}$, respectively. The C/N ratio was 12.01, the concentrations of Cu and Zn were 27.8 and 270 mg $kg^{-1}$, and the concentrations of lignin, cellulose, and hemicellulose were 530.7, 188.1, and 57.4 g $kg^{-1}$, respectively. The values of the other chemical and microbiological parameters of *A. dealbata* vermicompost samples (averaged per sampling time) are included in Table A1 (Appendix A).

The application of a PCA analysis of the *A. dealbata* substrate and vermicompost allowed us to identify four Principal Components (PCs) that explained more than 90% of the total variance. The first principal component (PC1) alone explained 61.5% of the variance. The loadings (i.e., the correlations) of each parameter on the four PCs are presented in Table 1. Almost all parameters were correlated with PC1. The variables with positive correlations included the moisture content and the concentrations of several elements such as N, P, S, and others. By contrast, electrical conductivity, organic matter content, basal respiration, and the concentrations of C, B, and Cl were negatively correlated with PC1 (Table 1). Microbial C, pH, and potassium concentration were positively correlated with PC2. Microbial N was negatively correlated with PC3, whereas K was positively correlated with PC3. Only one parameter (Mo concentration) was correlated with PC4 (Table 1).

**Table 1.** PCA analysis of *A. dealbata* vermicomposting process. The variation explained by four principal components (PC1 to PC4) and their eigenvalues are shown. The loadings of measured parameters on the four PCs are also shown. PCA was computed using a correlation matrix. Parameters strongly correlated with PCs (i.e., loadings > 0.55 or loadings < −0.55) are marked in **bold**.

| | **PC1** | **PC2** | **PC3** | **PC4** |
|---|---|---|---|---|
| Explained variation (%) | 61.5 | 15.3 | 8.1 | 5.4 |
| Eigenvalue | 12.9 | 3.2 | 1.7 | 1.1 |
| **Variable loadings** | | | | |
| pH | 0.47 | **0.64** | −0.45 | 0.03 |
| Electrical Conductivity | **−0.89** | −0.25 | 0.31 | −0.00 |
| Moisture content | **0.90** | 0.23 | −0.14 | −0.14 |
| Organic Matter content | **−0.87** | 0.36 | −0.00 | −0.00 |
| N total concentration | **0.89** | 0.31 | −0.18 | −0.20 |
| C total concentration | **−0.78** | 0.54 | −0.04 | 0.14 |
| B total concentration | **−0.83** | 0.14 | 0.28 | −0.13 |
| Ca total concentration | **0.83** | 0.45 | 0.25 | −0.07 |
| Cl total concentration | **−0.95** | −0.13 | 0.16 | 0.12 |
| Cu total concentration | **0.90** | −0.37 | 0.06 | 0.15 |
| Fe total concentration | **0.93** | −0.34 | 0.07 | 0.05 |
| K total concentration | −0.07 | **0.73** | **0.60** | 0.15 |
| Mg total concentration | **0.71** | 0.55 | 0.35 | −0.22 |
| Mn total concentration | **0.95** | 0.12 | 0.15 | −0.10 |
| Mo total concentration | 0.42 | −0.20 | 0.34 | **0.74** |
| P total concentration | **0.94** | 0.13 | 0.29 | −0.00 |
| S total concentration | **0.95** | 0.08 | 0.25 | −0.02 |
| Zn total concentration | **0.86** | −0.39 | 0.05 | 0.23 |
| Basal Respiration | **−0.92** | 0.24 | 0.12 | −0.02 |
| Microbial biomass C | −0.07 | **0.73** | −0.23 | 0.51 |
| Microbial biomass N | 0.24 | 0.16 | **−0.58** | 0.24 |

After plotting the *A. dealbata* and vermicompost samples on the PC1–PC2 axes, we observed that the samples were arranged along PC1 as a function of time (Figure 1): the PC1 scores for $t_0$ samples were below −1.5, the scores for $t_{14}$ samples were around −1, the scores for $t_{28}$ and $t_{42}$ were around 0.5, and the scores for $t_{56}$ samples were higher than 1. Considering these values and the parameters correlated with PC1, we interpreted that this axis provides information about the "vermicompost maturity". Regarding PC2, the samples from $t_0$ and $t_{56}$ had the lowest values, whereas the samples from the intermediate vermicomposting steps had the highest values. Given the correlation between microbial C biomass and PC2, we find that this axis indicated the "size of the bacterial community". The samples tended towards lower values of PC3 over the sampling time (Figure A1, Appendix A), whereas the values of PC4 are similar for all sampling times except for the t28 samples, which yielded lower values (Figure A1, Appendix A).

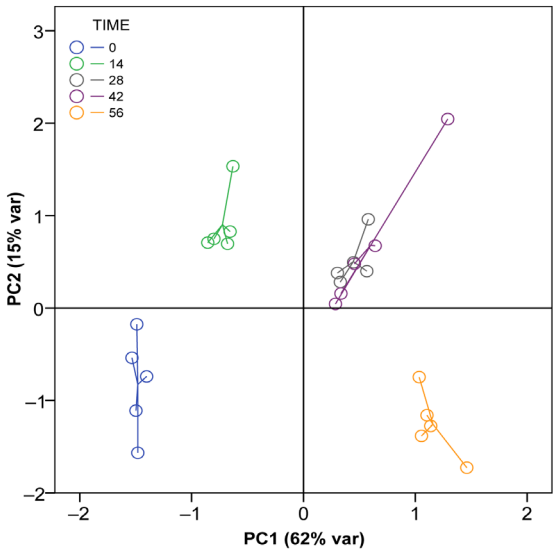



**Figure 1.** PCA results. *A. dealbata* samples collected at different times during vermicomposting are plotted in the space determined by the first and second principal components (PCs). Each empty dot represents one sample. Lines connect each sample with the group centroid. Different colours indicate different sampling times.

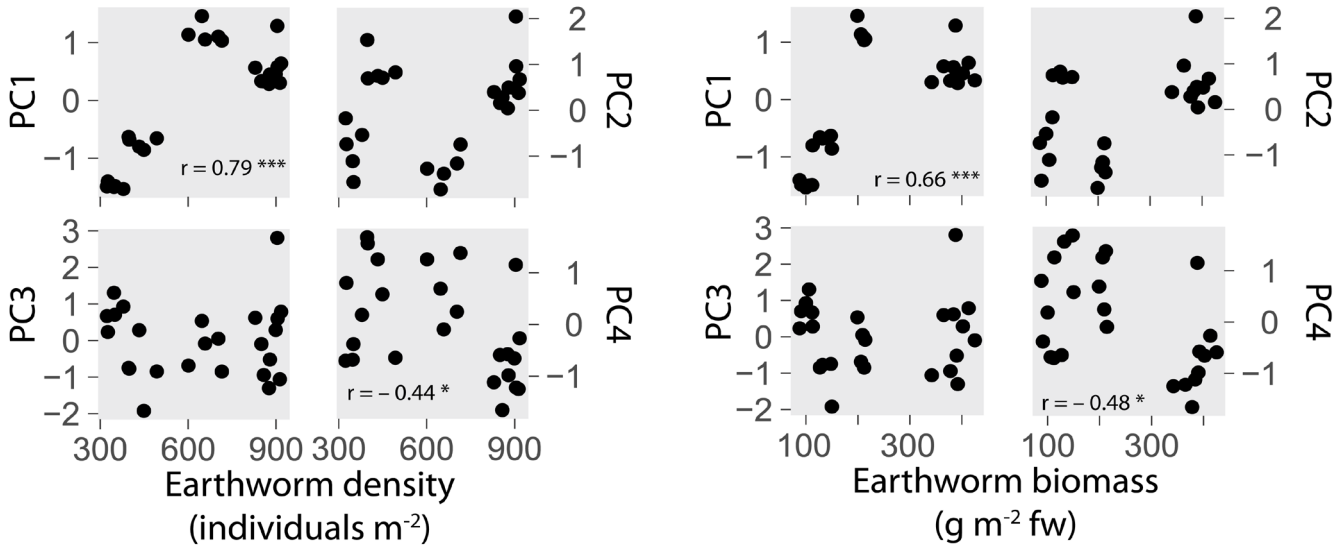
### *3.2. Changes in Earthworm Population*

The population of earthworms in the vermireactor varied significantly throughout the experiment (Table 2). The total earthworm density and biomass increased steadily from the beginning of the experiment. The maximum density (877 ± 34 individuals $m^{-2}$) was reached on day 28 and remained stationary until day 42. The maximum earthworm biomass (404.2 ± 15.2 g $m^{-2}$) was recorded on day 42. Both parameters then decreased until day 56 (Table 2).

**Table 2.** Variation in earthworm population during the vermicomposting of *A. dealbata*. The results of ANOVA (F and *p*-values) for the effects of the factor *time* are shown. The average ± standard deviations of each parameter for each sampling time are also shown. Different superscript letters indicate significant differences between sampling times, according to Tukey post hoc tests.

| | $F_{(4,20)}$ | *p*-Val | Day 0 | Day 14 | Day 28 | Day 42 | Day 56 |
|---|---|---|---|---|---|---|---|
| Earthworm density (individuals $m^{-2}$) | 254.9 | $7.5\ 10^{-17}$ | $345^{d} \pm 23$ | $434^{c} \pm 40$ | $877^{a} \pm 34$ | $889^{a} \pm 27$ | $665^{b} \pm 46$ |
| Earthworm biomass (g $m^{-2}$) | 493.3 | $1.13\ 10^{-19}$ | $99.4^{e} \pm 10$ | $134.2^{d} \pm 15.6$ | $371.9^{b} \pm 18.9$ | $404.2^{a} \pm 15.2$ | $208.2^{c} \pm 5.8$ |

The earthworm population parameters (density and biomass) and principal component PC1 were highly significantly correlated. These parameters were also negatively correlated with PC4 (Figure 2).




(**a**) (**b**)

**Figure 2.** Correlation between earthworm population and vermicompost parameters. (**a**) Scatter plot of total earthworm density (individuals $m^{-2}$) vs. PC1 to PC4 scores; (**b**) scatter plot of total earthworm biomass (g $m^{-2}$ fw) vs. PC1 to PC4 scores. The Pearson's correlation index (r) is shown in those cases of significant linear correlations. *: *p*-value < 0.05, *** *p*-value < 0.001.

### *3.3. Phytotoxic Effects before and after Vermicomposting*

The results show that the fresh *A. dealbata* material was phytotoxic. Thus, the germination percentage of *L. sativum* seeds in treatments AD5 and AD10 was around 80%, whereas the percentage of germination in the control was around 97% (Table 3). The differences in root elongation between the control and treatments with *A. dealbata* were clearer: in the control, the average radicle length was slightly below 7 cm, whereas in AD5 and AD10 the average length was around 2 cm (Figure 3). By contrast, the treatments with mature vermicompost (VC5 and VC10) yielded similar germination percentages and radicle elongation rates as the control (Table 3 and Figure 3).

**Table 3.** Variation in the germination percentage (after 7 days) of *L. sativum* seeds treated with water (C), *A. dealbata* extracts (AD5 and AD10), and vermicompost extracts (VC5 and VC10). The results of ANOVA (F and *p*-values) regarding the effects of the factor *treatment* are shown. The average ± standard deviation of germination percentage in each treatment are also shown. Different superscript letters indicate significant differences between treatments, according to Tukey post hoc tests.

| | $F_{(4,10)}$ | *p*-Val | C | AD5 | AD10 | VC5 | VC10 |
|---|---|---|---|---|---|---|---|
| Germination percentage (%) | 4.63 | 0.023 | $96.7^{a} \pm 2.9$ | $81.7^{b} \pm 5.8$ | $78.3^{b} \pm 5.8$ | $88.3^{a,b} \pm 7.6$ | $93.3^{a,b} \pm 7.6$ |

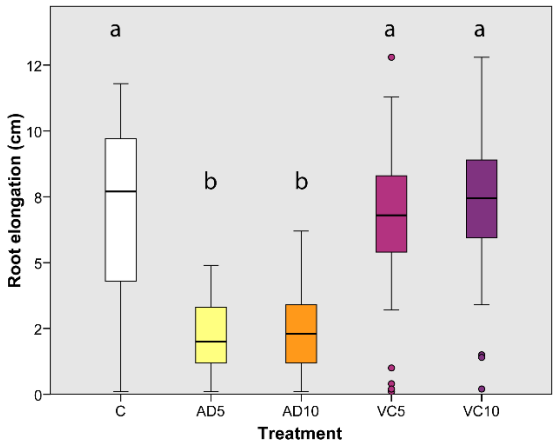



**Figure 3.** Boxplot of data obtained in the radicle elongation test with *L. sativum*. Treatments with extracts of fresh *A. dealbata* biomass are yellow and orange, whereas treatments with extracts of mature vermicompost are light and dark purple. Each box corresponds to the inter-quartile range, and the band inside the box denotes the median for radicle elongation in each treatment. Different letters indicate significant differences between treatments as indicated by Tukey post hoc test.

## 4. Discussion

We performed a pilot-scale study to assess the feasibility of vermicomposting as a way of managing and valorising the biomass of the invasive tree *A. dealbata*. The earthworm *Eisenia andrei* was able to grow and reproduce in this substrate, with a sustained increase in the number of individuals and earthworm biomass until day 28, followed by a plateau between days 28 and 42. After that point, the number of earthworms and their total biomass decreased until the end of the experiment. This demographic pattern is typical of populations growing in closed systems (i.e., systems without any matter exchange with any part outside the system), where populations grow steadily until the point when the consumption of all the available resources or the accumulation of wastes provokes a decrease in the population (e.g., [29]). Our results show a continuous reduction in hemicellulose (from 13.3% to 5.7%) and cellulose (from 21.7% to 18.8%) and an increase in lignin (from 38.8% to 53.2%) in the substrate during vermicomposting, which indicates the consumption of the more labile organic fractions by the earthworms and an enrichment in recalcitrant, less-palatable materials. Similarly, high concentrations of lignin and polyphenols in the leaf litter of *Acacia auriculiformis* were suggested to be responsible for the high earthworm mortality during the vermicomposting of this litter [30]. Other researchers found that the increase in N compounds and the decrease in the C:N ratio (depending on the residues, changes of C:N ratio from 40 to 20) negatively affected the growth and reproduction of *Eisenia fetida* during the vermicomposting of diverse organic residues [31]. However, it seems that the C:N ratio is not the limiting factor for *E. andrei,* because (i) the population in our experiment grew while the C:N ratio decreased from 19.9 to 12, and (ii) in another vermicomposting study with *E. andrei*, the C:N ratio of the substrate stayed at values around 12 (11.5 at day 0 and 12.9 at day 42) and the earthworm population and biomass increased steadily with no signs of population stagnation [25].

In addition to the relative changes in the hemicellulose, cellulose, and lignin proportions mentioned above, the main trends observed during the vermicomposting of *A. dealbata* biomass included reductions in the EC; OM content; concentrations of B, C, and Cl; and basal respiration, and increases in moisture content and in the concentrations of N, Ca, Cu, Fe, Mg, Mn, P, S, and Zn. Similar processes have been documented during the vermicomposting of other substrates [23–25] and are mainly related to i) rapid C mineralisation throughout the vermicomposting process (especially of the most labile fractions of the cell walls), which involves the concentration of other elements, and ii) the

reduction in the concentration of soluble ions due to leaching, precipitation into non-mobile salts, or immobilisation by microorganisms [24,32]. Other observed changes included an increase in pH and, especially, the microbial biomass of C during the intermediate stages of vermicomposting (day 14) and a decrease in these parameters until the final product. The observed variation in pH during vermicomposting (from acid values in the fresh material to more basic values in different vermicomposting stages) contrasts with the most common changes observed in the literature (progressive acidification during vermicomposting; see [33–35] and references therein), but is congruent with the changes observed during the aerobic composting of *A. dealbata* biomass [36]. While the acidification of the substrate during vermicomposting has been explained by the mineralisation of N and P and the formation of organic acids during the bioconversion of organic material [35], other authors have proposed that the increase in the pH in *A. dealbata* after composting was due to $NH_3$ formation and the degradation of organic acids [36].

The final product was stabilised organic matter, as indicated by the decrease in the microbial biomass of C and the basal respiration, as well as the decrease and stabilisation of the C:N ratio. Moreover, the vermicompost obtained met the EU-criteria for solid, nitrogen-rich organic fertilisers [37], i.e., solid and of organic origin; an N concentration above 2.5%; an organic carbon content higher than 15%; and concentrations of Cu and Zn below 300 and 800 mg $kg^{-1}$, respectively.

*A. dealbata* is known to possess secondary compounds such as phenolics, triterpenes, or alkaloids with allelopathic effects [7,10,11]. Thus, the use of *A. dealbata* as an organic fertiliser could be hampered by the presence of these phytotoxic compounds in the vermicompost. We conducted an ecotoxicological test with *Lepidium sativum*, a plant which has been widely used in this kind of study (e.g., [38]). Our results indicate that vermicomposting eliminated the phytotoxicity of the fresh material. Moreover, radicle elongation was a parameter with a higher sensitivity than the seed germination percentage. Similar reductions in phytotoxicity were obtained after the composting of the vermicompost of diverse organic residues such as manure, biosolids, and urban or food wastes [39–42]. Although we did not quantify the secondary compounds present in the fresh *A. dealbata* biomass and the vermicompost, we assume that our results indicated that the phytotoxic compounds have been degraded during the vermicomposting process. Accordingly, previous research found that vermicomposting with *E. andrei* totally degraded the polyphenols present in Scotch broom biomass [25] and reduced the concentrations of these phytotoxic compounds in grape marc [23]. Moreover, a recent study indicated that vermicompost derived from different types of organic waste can contain high amounts of different extracellular catalytic enzymes that are even capable of degrading organophosphorus pesticides [24].

## 5. Conclusions

We have performed a pilot-scale assay of vermicomposting as an alternative for the management of the invasive tree *Acacia dealbata*. We have found that *E. andrei* is able to grow and process the biomass of this tree and produce a stabilised material after 56 days. The obtained vermicompost meets the EU requirements for solid organic fertilisers. Our results also indicate that vermicomposting eliminated the phytotoxicity of *A. dealbata* biomass.

Some value chains have been proposed as a way to promote the control and eradication of the invasive tree *A. dealbata.* Overall, our findings indicate that vermicomposting with *E. andrei* is a feasible alternative for the conversion of this organic residue into a valuable fertiliser, thereby contributing to decreasing the impact of alien species on native biodiversity.

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preparation, C.Q.-S.; writing—review and editing, J.D., L.A.M. and C.Q.-S.; supervision, J.D.; project administration, J.D.; funding acquisition, J.D. All authors have read and agreed to the published version of the manuscript.

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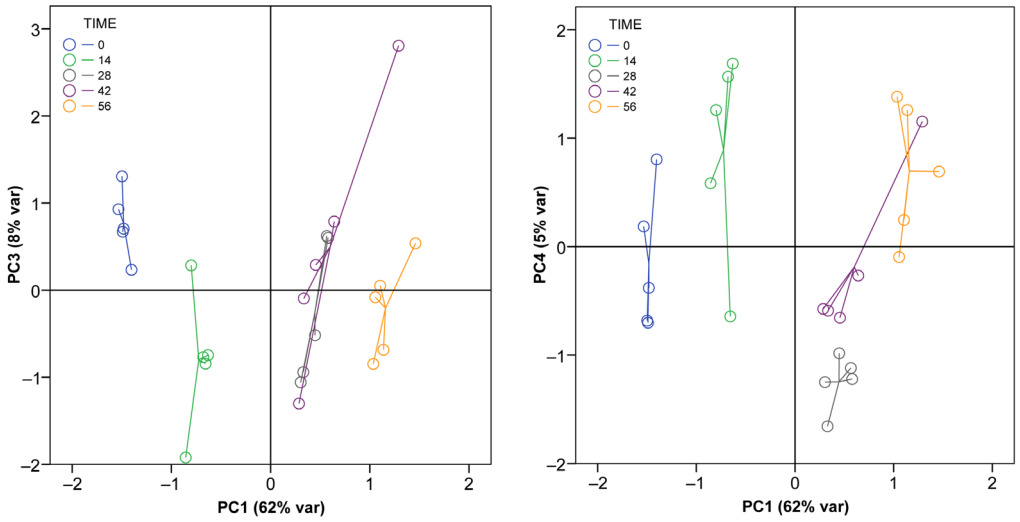
## Appendix A

**Table A1.** Parameters of *A. dealbata* biomass at different points of the vermicomposting process. The values were averaged per sampling time ($n$ = 5; except for fibers, $n$ = 3).

| Variable (Unit) | Time (Days) | | | | |
|---|---|---|---|---|---|
| | **0** | **14** | **28** | **42** | **56** |
| Moisture (%) | 64.23 | 72.08 | 78.63 | 80.7 | 79.56 |
| pH | 5.14 | 7.26 | 7.15 | 6.63 | 6.42 |
| Electrical Conductivity (mS $cm^{-2}$) | 752.4 | 370.8 | 259 | 263.6 | 211.8 |
| Organic Matter (%) | 96.64 | 96.38 | 92.89 | 93.41 | 89.12 |
| Total C (g $kg^{-1}$) | 506.2 | 516.3 | 489.2 | 493.6 | 471.1 |
| Total N (g $kg^{-1}$) | 25.5 | 33.3 | 40.8 | 41.3 | 39.2 |
| C:N | 19.9 | 15.49 | 11.99 | 11.96 | 12.01 |
| Total B (mg $kg^{-1}$) | 62.39 | 43.56 | 37.22 | 28.19 | 15.71 |
| Total Ca (g $kg^{-1}$) | 4.03 | 5.84 | 8.24 | 8.29 | 7.35 |
| Total Cl (g $kg^{-1}$) | 6.86 | 4.22 | 0.89 | 0.98 | 0.56 |
| Total Cu (mg $kg^{-1}$) | 2.7 | 3.6 | 12.3 | 13.6 | 27.8 |
| Total Fe (g $kg^{-1}$) | 0.11 | 0.12 | 0.57 | 0.57 | 1.07 |
| Total K (g $kg^{-1}$) | 1.82 | 1.96 | 1.78 | 2.23 | 1.38 |
| Total Mg(g $kg^{-1}$) | 0.92 | 1.16 | 1.74 | 1.78 | 1.34 |
| Total Mn (mg $kg^{-1}$) | 89.3 | 119.1 | 216.8 | 205.3 | 229.9 |
| Total Mo (mg $kg^{-1}$) | 0.8 | 0.9 | 0.71 | 0.92 | 1.14 |
| Total P (g $kg^{-1}$) | 0.61 | 0.76 | 1.23 | 1.39 | 1.42 |
| Total S (g $kg^{-1}$) | 0.26 | 0.36 | 0.74 | 0.95 | 0.94 |
| Total Zn (mg $kg^{-1}$) | 11.5 | 15.0 | 85.8 | 122.0 | 269.6 |
| Basal respiration (μg $CO_2$ $g^{-1}$ dw $h^{-1}$) | 701.5 | 594.5 | 391.8 | 320.1 | 127.6 |
| Microbial biomass C (g $kg^{-1}$) | 9.55 | 24.08 | 12.44 | 16.12 | 10.81 |
| Microbial biomass N (g $kg^{-1}$) | 4.26 | 5.72 | 5.60 | 5.37 | 5.84 |
| Lignin (g $kg^{-1}$) | 388.4 | 519.2 | 518.5 | 544.9 | 530.7 |
| Cellulose (g $kg^{-1}$) | 217.1 | 219.9 | 209.1 | 192.1 | 188.1 |
| Hemicellulose (g $kg^{-1}$) | 133.1 | 94.7 | 71.6 | 71.3 | 57.4 |




(**a**) (**b**)

**Figure A1.** Plots of *A. dealbata* biomass and vermicompost samples: (**a**) PC1 vs. PC3 scores; (**b**) PC1 vs. PC4 scores. Each empty dot represents one sample. Lines connect each sample with the group centroid. Colours indicate different sampling times.

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