

Article

Impact of Fertilization with Cattle Slurry in a Poplar Short Rotation Coppice on Mass Balance of Nutrients and Biomass Productivity

Abel Rodrigues ^{1,2,*} , Sofia Pereira ³ , Alberto Azevedo Gomes ¹, José Louzada ⁴ , Alexandre B. Gonçalves ⁵ , Teresa Fonseca ⁴ , Amélia Castelo-Branco ¹, Paula Castro ³ , Helena Moreira ^{3,6,7}  and Regina Menino ¹ 

¹ Instituto Nacional de Investigação Agrária e Veterinária (INIAV), 2780-157 Oeiras, Portugal; amazevedogomes@gmail.com (A.A.G.); regina.menino@iniav.pt (R.M.)

² IDMEC, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal

³ CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho, 1327, 4169-005 Porto, Portugal; sapereira@ucp.pt (S.P.); plcastro@ucp.pt (P.C.); helena.moreira@cibio.up.pt (H.M.)

⁴ Centre for Research and Technology of Agro-Environmental and Biological Sciences, CITAB, Inov4Agro, Universidade de Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal; jlousada@utad.pt (J.L.); tfonseca@utad.pt (T.F.)

⁵ CERIS, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal; alexandre.goncalves@tecnico.ulisboa.pt

⁶ Estação Biológica de Mértola (EBM), CIBIO, Praça Luís de Camões, Mértola, 7750-329 Mértola, Portugal

⁷ BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

* Correspondence: abel.rodrigues@iniav.pt

Abstract

The incorporation of cattle slurry in soil in short-rotation-cycle poplar cultivations can be a win–win strategy, insofar as a main feedstock derived from local intensive dairy cattle breeding can be used as a natural fertilizer and in bioenergy produced in the same region. The circularity of this process can contribute to boosting local socio-economic value. In this context, this work involved the installation of a poplar SRC plantation with a density of 5330 trees ha^{−1} in a 4000 m² moderately fertile flat site, which was formerly used as a vineyard. Mechanical dosages of slurry of 0, 26.6, 53.2, and 106.5 Mg ha^{−1}, designated as treatments T0, T1, T2, and T3, were applied three times per year during 2019, 2020, and 2021. The variables quantified were related to plant growth, biomass productivity and mass balances of K, P, Cu, Zn, Mg, and N, and organic matter in the whole soil, plant, and slurry system during the first rotation cycle. For treatments T0 and T1, all these seven chemical components showed positive balances in the system, with cumulative demand by soil and biomass being higher than cumulative supply by slurry. Negative balances occurred for P with T2 and T3 and for Zn with T3, so that an overall condition of nutrient saturation of the whole system was not achieved. A no-slurry application, or at most a moderate application equivalent to T1, in the second rotation cycle should therefore be prescribed to allow a nutrient equilibrium status to be achieved through internal seasonal recycling mechanisms. The biomass average productivities ranged from 6.1 to 11.8 Mg ha^{−1} y^{−1}, peaking under treatment T2, and are within the typical values for a first rotation cycle for poplar SRCs. The biomass fuel quality was not affected by the slurry treatments. A good performance of plant total height and growth in diameter at breast height suggested that poplar trees were not stressed by the applied slurry. Only treatment T1 could assure that cattle CO₂-eq methane emissions were overall equilibrated by the carbon sequestration from poplar cultivation, with an absence of climatic-warming impacts. Treatments T2 and T3 could only partially minimize that impact, which would always exist. Globally, this



Academic Editor: Norman Q. Arancon

Received: 21 October 2025

Revised: 14 November 2025

Accepted: 19 November 2025

Published: 22 November 2025

Citation: Rodrigues, A.; Pereira, S.; Gomes, A.A.; Louzada, J.; Gonçalves, A.B.; Fonseca, T.; Castelo-Branco, A.; Castro, P.; Moreira, H.; Menino, R. Impact of Fertilization with Cattle Slurry in a Poplar Short Rotation Coppice on Mass Balance of Nutrients and Biomass Productivity. *Appl. Sci.* **2025**, *15*, 12403. <https://doi.org/10.3390/app152312403>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

site-specific analysis showed that, under moderately fertile conditions, controlled cattle slurry fertilization of poplar SRC cultivations, which would assure a long-term steady-state equilibrium, can be a viable option to contribute to decentralized production of bioenergy in rural communities.

Keywords: bioenergy; cattle slurry; circularity; nutrients; poplar; short rotation coppice

1. Introduction

Short-rotation coppices (SRCs) are high-density agroecosystems that aim to produce woody biomass by intensively cultivating forest species managed as coppices through mechanized operations such as weeding, planting, and harvesting. In Europe, poplar is a species that is very appreciated as an SRC due to its favorable characteristics, such as high growth rates, high plasticity to microclimates and geographies, high genetic potential, and low requirements for irrigation, herbicides, and fertilizers [1–4]. The entire sequencing of the poplar genome has been carried out, and genetically engineered plants display high productivity and phytoremediation potentials reflecting enhanced overexpression of regulating genes [5,6]. The usual biomass productivity of SRC cultivations ranges between 10 and 24 Mg of dry matter per year and hectare under 1- to 5-year rotation cycles and potential lifetimes of 5–7 cycles [2,3,7]. The ability of poplar species to produce biomass for bioenergy and to accumulate relatively high concentrations of inorganic nutrients, including heavy metals, by phytoextraction has been clearly identified [6,8,9]. Due to their rapid growth, SRC poplar plants actively promote and share horizontal and vertical resource pools, light, water, and nutrients in the agroecosystems in which they are fully or partially integrated, e.g., intercropping, tree–animal systems, or windbreaks. SRC cultivations should be implemented in marginal soils, corresponding to moderate to poor site conditions not adequate for food crops and where some fertilization and irrigation are needed, e.g., in Mediterranean areas, for the effects of upgrading biomass productivity and financial feasibility [1,3,8,10–13]. Cattle slurry is traditionally considered a valuable natural organic fertilizer, especially since it was a main method of soil enrichment before the 20th century. In Portugal, intensive dairy cattle breeding has a high social and economic impact, particularly in northern regions. Fertilization of SRC cultivations with cattle slurry reduces the substantial operational costs of chemical fertilizers and enhances environmental sustainability through the optimization of circular mineral recycling.

Land application of slurry as a natural fertilizer in SRC cultivation will induce increases in energy ratios of cultivation, defined as the ratio between the energy contained in biomass and the energy inputs necessary to produce the biomass feedstock, and will deliver yields comparable to those of traditional fertilizers [14–16]. The use of marginal land for these cultivations can be facilitated through an increase in soil OM, with potential improvements in the cation exchange capacity or water holding capacity [10,11,17].

Slurry can present high levels of macronutrients, such as nitrogen (N), phosphorus (P), and potassium (K), of micronutrients of heavy metals such as copper (Cu), zinc (Zn), and manganese (Mn), and of organic matter (OM), constituting a valuable organic fertilizer for soils by increasing their content of organic matter and nutrients, while improving their structure [12,16,18]. In this way, the application of slurry can be a good alternative or complement to mineral fertilizers in agroforestry systems. Heavy metals present in livestock manure, although fundamental for plant physiological dynamics, can be toxic to crops if present in concentrations above certain thresholds [5,12,19]. Furthermore, the application of natural OM can contribute to metal immobilization in soils through chemical

complexation mechanisms [12]. In soil, labile carbon components from cattle slurry may trigger microbial denitrification from nitrogen present in the organic fertilizer, with the release of nitrous oxide gas (N_2O), a potent greenhouse gas. These emissions counterbalance carbon sequestration in soil and avoid carbon emissions from the manufacture of chemical fertilizer, which is replaced by the slurry emission. A fraction of about 65% of nitrogen in cattle slurry corresponds to ammonia (NH_3), which is lost by volatilization after application to the soil surface. On the other hand, organic fertilizers, in general, display low C/N ratios, so that mineralization prevails over microbial immobilization of nitrogen and plant-available N increases in soil [20,21]. Environmental problems related to ground-water pollution may arise from slurry application when highly mobile compounds in soil, such as nitrates, ammonium, phosphates, or heavy metals, accumulate due to prolonged applications or if nutrient loads exceed soil saturation capacity. A reasonable prescription of slurry application in SRC agroecosystems must account for these environmental and productive effects and counter-effects [6,18,22].

Using slurry in poplar SRC cultivations for biomass production also helps sequester excessive inorganic components from human and animal food chains, thereby reducing their toxicity risks. Examples of the potential of these cultivations for removing soil contaminants are as follows: (i) Poplar behavior as a phreatophyte species apt to nitrate phytoremediation through high evapotranspiration rates from nitrate-contaminated saturated soil layers below shallow water tables. For example, poplar species can be riparian components of agroforestry, stabilizing degraded agricultural streambanks while growing rapidly. (ii) Its behavior in phytodegradation of pesticides by enzymatic hydrolysis and dealkylation of the herbicide atrazine to deliver less toxic metabolites, which are lifted and degraded in plant tissues [6,10,19,23–25]. (iii) Phytodegradation and enzymatic degradation by dehalogenation and detoxification of petroleum hydrocarbons [19,23–25]. (iv) Phytoextraction or phytoaccumulation of toxic metals by root absorption and translocation to shoots and deposition in cell walls, cell membranes, and vacuoles or other metabolically inactive parts of plant tissues. External metabolization by hydrolyzing large and complex soil contaminants, which cannot be taken up by plants, into simpler molecules can be carried out by enzymes excreted into the soil close to the rhizosphere. These smaller molecules are more susceptible to assimilation by plant tissues [6,19,23–26]. (v) Poplar species have also been associated with the removal of volatile compounds from the field through phytovolatilization mechanisms due to transpiration for bark or leaves, which can act on volatile organic compounds such as trichloroethylene or on heavy metals by conversion into volatile chemical compounds [5,6,19,27]. Although poplar species are not considered as real hyperaccumulators of hazardous chemical compounds, they are preferred in commercial phytoremediation due to their perennially fast growth, biomass productivity, and their developed root system, which can penetrate deep into the ground. Another main characteristic of poplar SRC cultivations is their variability, both in biomass production and in phytoremediation capabilities, with differences in microclimate, soil, and living organisms and microbes among sites. Therefore, site-specific approaches to their implementation are useful, combining pre-testing and field experimentation of different poplar clones interacting with different management and environmental conditions, to identify potential better-performing clones for possible alternative commercial upscaling [5,6,10,19].

Under the above context, this work aims to quantify the impact of the application of bovine slurry as a natural fertilizer in a poplar SRC field site, considering plant biometry, biomass productivity and quality, and soil–plant relationships through mass balances of nutrients in the main components of the agroecosystem. Multivariate analysis allowed the identification of a set of 14 main soil, biometric, slurry, and biomass variables for the typification of the slurry treatment. Our ultimate goal was to contribute to the evaluation

of the environmental sustainability and financial feasibility of a solution to cattle slurry, resulting from intensive dairy breeding based on its application as fertilizer for poplar SRC cultivations.

2. Materials and Methods

2.1. Location and Experimental Settings

This research lasted for three years between 2019 and 2021, beginning with the installation of a poplar SRC plantation with a poplar clone i214 (*Populus deltoides* × *Populus nigra* [*Populus* × *euroamericana* (Dode) Guinier]) with a density of 5330 trees ha⁻¹ in a field site of 4000 m² in the Penafiel municipality in Northern Portugal (41°12'6" N, 8°18'17" W) in March 2019, until the harvesting of this plantation in November 2021 (Figure 1).

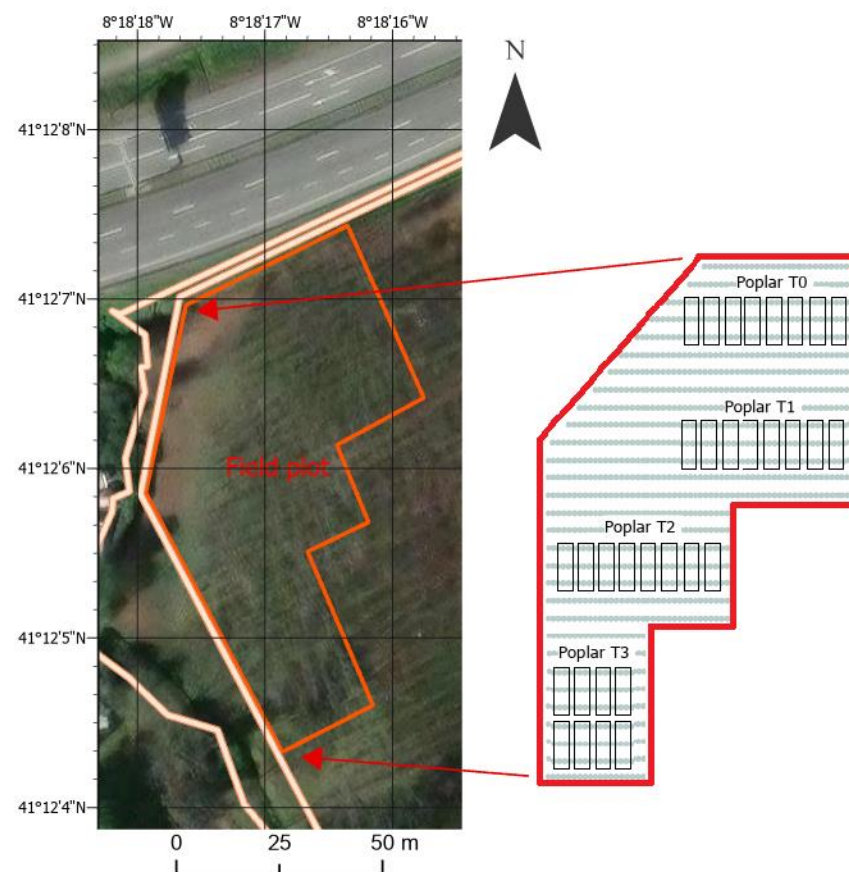


Figure 1. Map and diagram of the experimental site displaying the T0 to T3 plots.

The isolated flat site with the shape of a 9-edge polygon corresponded to land formerly used as a vineyard, with anthrosol sandy-loam-textured soil. The site was subjected to mechanical application of a seasonal slurry from bovine livestock each year in spring, summer, and autumn, totaling nine applications over the study period. These treatments aimed to evaluate the fertilizer potential of this feedstock for poplar growth and biomass productivity, as well as to assess the potential of this agroforestry system for phytoremediation and storage of potentially excessive input nutrients. In each application, four doses of slurry were mechanically applied at 0 Mg ha⁻¹, 26.6 Mg ha⁻¹, 53.2 Mg ha⁻¹, and 106.5 Mg ha⁻¹ to the soil, along plant plot lines, corresponding, one-to-one, to four treatments, T0, T1, T2, and T3. These application rates supplied N dosages of 0 kg ha⁻¹, 85 kg ha⁻¹, 170 kg ha⁻¹, and 340 kg ha⁻¹, respectively. Chemical and physical analyses of soil, slurry, and biomass, along with measurements of biomass productivity and plant biometry, were carried out across the slurry treatment plots to establish mass balances

of nutrients and identify other soil–plant interactions across those treatments. Random subsets of 35, 72, 64, and 44 trees, across the treatments T0, T1, T2, and T3 (variable TREAT) were selected for biometric measurements in 2019, 2020, and 2021. The measured variables were plant height (variables TH19, TH20, and TH21) using poles and tapes, and trunk diameter at a breast height of 1.3 m (variables DBH19, DBH20, and DBH21) using digital calipers. After the harvest, dry biomass productivity (variable BioPr) was estimated by weighing the whole aerial sliced biomass of six trees in each plot, which was converted to dry weight. From the measured plant heights and breast trunk diameters, four derived variables were calculated to quantify the absolute growth increases across the treatments in plant TH and DBH between 2020 and 2019, and between 2021 and 2020, which were Inc2019TH, Inc2019DBH, Inc2120TH, and Inc2120DBH, respectively. Another four variables used, derived from the measured values of plant height and breast-height trunk diameter, were related to the relative percent growth increases, defined by the dimensionless ratios between absolute increases in the TH and DBH between 2020 and 2019, and between 2021 and 2020, and the initial TH and DBH values in these biennial periods. These variables were Increl2019TH, Increl2019DBH, Increl2120TH, and Increl2120DBH, respectively.

2.2. Chemical and Physical Analysis of Soil and Cattle Slurry

The applied cattle slurry was analyzed once a year to ensure that its contents of chemical elements ranged within the limits allowed for agricultural soils, according to EN standards in the scope of usual procedures of national and EU environmental legislation. The chemical and physical variables analyzed in the slurry were moisture and dry matter, pH (H₂O), electric conductivity, total nitrogen (N) [28], organic matter (OM), total phosphorus (P), total potassium (K), total calcium (Ca), total magnesium (Mg), total boron (B), total sodium (Na), total copper (Cu), total zinc (Zn), total nickel (Ni), total chromium (Cr), total lead (Pb), and chlorine. The nomenclature used for slurry chemical variables, varying with each slurry treatment, in this work attached the prefix sl to the symbol of each chemical element, delivering 15 slurry variables as follows: slOM, slP, slK, slN, slCa, slMg, slNa, slCu, slZn, slN, slCr, slPb, slBo, slNi, and sldose for the slurry doses.

Soil samples were taken in 2019, 2020, and 2021, with vertical soil probes at the top 30 cm layers, totaling 28 samples that corresponded to a composite sample per treatment and year, with duplicates in 2019 and triplicates in 2020 and 2021. It was assumed that the soil chemical composition in the top 30 cm depth layers was representative of the bulk of soil–plant interactions in a quasi-steady equilibrium, considering that litterfall is a transient condition with continuous fluctuations. The composite soil samples collected each year were dried at 40 °C until they reached a constant weight, sieved through a 2 mm mesh, and analyzed for pH, soil organic matter, N, P, K, Mg, Zn, Cu, Fe, and Mn. Soil pH was determined by suspending samples in solution by adding deionized water in a soil-to-water ratio of 1:2.5, and measuring it with a glass electrode pH meter after one hour of contact, with agitation. Soil organic matter was determined by wet digestion with the potassium dichromate oxidation method [29] and molecular absorption spectroscopy (MAS). Soil P and K were determined after extraction with ammonium lactate [30] and measured by flame photometry, while Mg was extracted with a solution of 1 M of ammonium acetate and measured by atomic absorption spectroscopy-flame (AAS-flame). As for Zn and Cu, these were extracted by EDTA and determined by AAS-flame. Total N was evaluated by the Kjeldahl method [28]. Soil bulk density was determined by the core method with a cylinder. The nomenclature adopted for chemical variables of soil in this work joined a prefix s for soil, with the symbol of the chemical element/property, and with the postfixes 19, 20, and 21 for the year. So, a total of 30 soil variables were generated from soil analysis as follows: spH19, spH20, spH21, sOM19, sOM20, sOM21, sN19, sN20, sN21, sK19, sK20,

sK21, sP19, sP20, sP21, sCu19, sCu20, sCu21, sFe19, sFe20, sFe21, sMn19, sMn20, sMn21, sZn19, sZn20, sZn21, sMg19, sMg20, and sMg21.

Woody biomass analysis was carried out in 2021 after harvest of the poplar plantation for the evaluation of the cumulative effects of each slurry treatment. The biomass samples were characterized in terms of proximate and ultimate elemental analysis, major elements K, P, and Mg, minor elements Cu and Zn, and high heating value (HHV). Biomass elemental analysis of C, N, H, and O was performed with a LECO CHN628 analyzer. The determination of K, P, and Mg in biomass was carried out with inductively coupled plasma atomic emission spectroscopy (ICP-AES). The biomass analysis of Cu and Zn was carried out with graphite furnace atomic absorption spectroscopy (GF-AAS). Proximate analysis of biomass for quantification of moisture, ash, volatiles (Vol), and fixed carbon (FC) was carried out with about 1 g of material, respectively, with fixed carbon determined by difference.

The nomenclature adopted for biomass chemical variables in this work attached the prefix *Bio* for biomass to the symbol of each chemical element/property. As such, a total of 15 biomass variables were obtained from the chemical analysis, as follows: BioFC, BioVol, BioMg, BioP, BioK, BioN, BioCu, BioFe, BioZn, BioMn, BioCa, BioNi, BioCr, BioPb, and BioNa.

2.3. Mass Balances and Statistical Analysis

The nutrients common to the soil, biomass, and slurry were K, P, Cu, Zn, Mg, and N, while OM was common only to soil and slurry. Cumulative mass balances in 2021 for each treatment, defined as the sum of nutrient content in the 0–25 cm topsoil layer in 2021 and biomass nutrients, subtracted by nutrients added by nine slurry applications in 2019, 2020, and 2021, delivered insights into the impact of slurry treatments on potential soil nutrient saturation and soil–plant interaction dynamics within the agroecosystem.

A correlation analysis was performed on the set of 75 biometric, slurry, soil, and biomass variables, considering the 4 slurry treatments as cases. Significant correlations were evaluated through *t* significance tests on elements of the Pearce correlation matrix for $p < 0.05$. A subset of 14 variables with significant correlations was considered as those highly directly or indirectly impacted and subjected to ANOVA modeling, using a general linear approach with repeated measures. This strategy enabled the evaluation of the significance of differences between least-square means or contrasts of the abovementioned plant biometric and soil variables, which were considered dependent variables directly or indirectly influenced by the independent categorical variable, which was the slurry treatment in the plantation. Principal component analysis (PCA) and factorial analysis were also applied to the subset of 14 variables after correlation and ANOVA calculations to distinguish groups of biometric, soil, and biomass variables that were very close to each other in the factorial plan, thus reflecting potential interactions between soil, plant, and slurry variables in this agroecosystem. Nonlinear Levenberg–Marquardt least-squares models were used to model the relationships between biomass production and plant DBH and TH. All statistical calculations were performed using Statistica 12 software.

3. Results and Discussion

3.1. Mass Balances of Nutrients and Organic Matter in Soil and Biomass

Mass balances (kg ha^{-1}) for the poplar plots with the four treatments were evaluated for the nutrients common to the top 0–25 cm layer of soil, slurry, and biomass, which were K, P, Cu, Zn, Mg, and N. Organic matter was common only to slurry and soil. The average apparent density of the sandy-loam-textured soil was 1.46 g cm^{-3} . From an agronomic perspective, the percentage contents of these common components of common nutrients in soil for each slurry treatment in 2019 were rated, according to the LQARS Portuguese standard, as high

to very high for K and P, moderate to moderately high for Cu and Zn, very high for Mg and total N, and moderate to high for OM. In 2021, the qualitative soil nutrient profiles were similar, with high contents of K, moderate to high contents of P, moderate contents of Cu and Zn, high to very high contents of Mg, very high contents of total N, and moderate to high contents of OM. These results allowed us to consider the soil as having a moderate to high fertility profile. The soil nutrient content and organic matter for 2019 and 2021 are shown in Tables 1 and 2, respectively. Analysis of bovine slurry showed that its composition ranged within the limits allowed for application in agricultural soils by the EU and national legislation. The chemical composition of the slurry was extended to hectare and year for the calculation of mass balances of nutrients in the slurry–plant–soil system. In 2019, sK19, sP19, and sCu19 ranged from 420 kg ha^{−1} (T0) to 945 kg ha^{−1} (T3), 450 kg ha^{−1} (T0) to 515 kg ha^{−1} (T1), and 16 kg ha^{−1} (T0) to 24 kg ha^{−1} (T3), respectively (Table 1). For sZn19, sMg19, sN19, and sOM19, the corresponding values ranged from 5.8 kg ha^{−1} (T0) to 13.1 kg ha^{−1} (T3), 690 kg ha^{−1} (T3) to 989 kg ha^{−1} (T1), 3650 kg ha^{−1} (T2) to 4198 kg ha^{−1} (T3), and 45,625 kg ha^{−1} (T0) to 65,700 kg ha^{−1} (T3), respectively. Only sK19 and sOM19 showed absolute ascending tendencies for the slurry dosage applied to the soil. For sP19, sCu19, and sZn19, a slight ascending tendency could be assumed, with T3 plots exhibiting the highest values of sCu19 and sZn19. sN19 showed an oscillating tendency amongst the four slurry treatments.

Table 1. Contents of nutrients and organic matter (kg ha^{−1}) in the top 0–25 cm layer of soil in 2019.

Treatment		sK19	sP19	sCu19	sZn19	sMg19	sN19	sOM19
T0	average	420	450.00	16	5.8	788	3650	45,625
	std	64	41.53	6.7	1.5	67	214	12,451
T1	average	489	514.65	20	9.5	989	4125	51,100
	std	39	157.27	1.8	1.6	91	2168	3335
T2	average	613	467.20	17.5	6.6	744	3577	60,225
	std	126	95.81	7.4	0.8	125	662	22,851
T3	average	945	500.05	24	13.1	690	4198	65,700
	std	38	43.65	3.7	1.2	51	382	2816

std—standard deviation.

Table 2. Contents of nutrients and organic matter (kg ha^{−1}) in the top 0–25 cm layer of soil in 2021.

Treatment		sK21	sP21	sCu21	sZn21	sMg21	sN21	sOM21
T0	average	648	392	13.8	2.1	353	3951	63,570
	std	184	17	3.9	0.3	56	627	7088
T1	average	1229	300	27.9	7.6	389	3790	69,958
	std	51	30	2.3	1	22	166	12,151
T2	average	1326	289	14	6	520	3679	83,343
	std	472	141	5.7	1.8	20	200	3195
T3	average	1349	230	15.5	9	543	4556	64,788
	std	182	27	1.7	1.6	14	2057	1661

std—standard deviation.

In 2021, the values of sK21, sP21, and sCu21 ranged from 648 kg ha^{−1} (T0) to 1349 kg ha^{−1} (T3), 230 kg ha^{−1} (T3) to 392 kg ha^{−1} (T0), and 13.8 kg ha^{−1} (T0) to 27.9 kg ha^{−1} (T1) (Table 2). The corresponding values of sZn21, sMg21, sN21, and sOM21 ranged from 2.1 kg ha^{−1} (T0) to

9 kg ha⁻¹ (T3), 353 kg ha⁻¹ (T0) to 543 kg ha⁻¹ (T3), 3951 kg ha⁻¹ (T0) to 4556 kg ha⁻¹ (T3), and 63,570 kg ha⁻¹ (T0) to 83,343 kg ha⁻¹ (T2), respectively. Only sK21 and sMg21 showed absolute ascending trends with the applied slurry dosage, with the former and the latter showing higher and lower values in comparison with 2019, respectively.

While sP21 showed a decreasing trend across the four slurry dosages, sCu21 and sN21 showed oscillating patterns, with the latter peaking under treatment T3. The variable sZn21 showed a slight increasing trend with the slurry doses. These soil nutrient contents reflected a cumulative effect due to the nine slurry treatments applied over three years, with overall inputs of sOM and sK, sP, sCu, sZn, sMg, sN, and sOM (kg ha⁻¹), and were calculated from the chemical composition of the slurry given in Table 3. From treatment T1 to T3, these variables ranged from 395 to 1582 kg ha⁻¹, 192 to 767 kg ha⁻¹, 0.6 to 2.5 kg ha⁻¹, 3.4 to 13.8 kg ha⁻¹, 108 to 431 kg ha⁻¹, 249 to 995 kg ha⁻¹, and 5992 to 23,966 kg ha⁻¹, respectively. For the total N contents of slurry dosages, it was assumed that 65% of total N was present as ammonia, which was volatilized by 50% after application to soil [31,32].

Table 3. Slurry total inputs of K, P, Cu, Zn, Mg, N, and OM (kg ha⁻¹) to soil and biomass due to the nine slurry treatments in 2019, 2020, and 2021.

Treatment	sK	sP	sCu	sZn	sMg	sN	sOM
T1	395	192	0.6	3.4	108	249	5992
T2	791	383	1.2	6.9	216	497	11,983
T3	1582	767	2.5	13.8	431	995	23,966

The annual soil inputs of P, N, and K from three slurry treatments ranged from 20 to 79 kg ha⁻¹, 83 to 332 kg ha⁻¹, and 55 to 219 kg ha⁻¹, which were within the range of nutrient application dosages recommended for poplar and willow SRCs. For example, Ceotto et al. [20], who, to our best knowledge, are amongst the few to address this issue of fertilizing poplar SRC crops with cattle slurry, worked on an i214 SRC poplar clone field trial in Northern Italy, in which cattle slurry was applied annually over a continuous period of four years, corresponding to two biennial rotation cycles. A total of 12 annual applications of slurry to the soil were carried out, wherein one half was treated with a dosage of 100 Mg ha⁻¹ and the other half was treated with a dosage of 200 Mg ha⁻¹. For the former, the N amounts were 190, 300, 490, 200, 180, and 80 kg ha⁻¹. The corresponding P amounts were 20, 30, 50, 15, 12, and 27 kg ha⁻¹. For the slurry dosage of 200 Mg ha⁻¹, double amounts of N and P were applied. The same authors also developed research on two other bioenergy cultivations of sweet sorghum [31] and giant reed [33], wherein fertilization with cattle slurry dosages of the same order of magnitude as those for the poplar SRC cited in [20] was applied annually during five-year periods. For a poplar SRC, under a three-year rotation cycle, Adebদিগি et al. [34], in an SRC field trial conducted in Tully, New York, reported annual chemical fertilizer dosages of 336 kg ha⁻¹, 112 kg ha⁻¹, and 224 kg ha⁻¹ for N, P, and K, respectively, over four-year periods. DiMatteo et al. [35], in a poplar SRC trial in Southern Italy, applied a unique top-dressing of N fertilization of 100 kg ha⁻¹, 31 kg ha⁻¹ of P, and 41 kg ha⁻¹ of K. For field plots of willow SRCs, Quaye and Volk [15], in the Northeastern USA, reported single N application rates of 150 and 200 kg ha⁻¹ at the beginning of a three-year rotation cycle, and a threshold of 350 kg ha⁻¹ y⁻¹ for N is mentioned as a conventional recommended application rate by Wang et al. [36]. Other technical guidelines for poplar SRCs recommend unique dosages between 180 kg ha⁻¹ and 200 kg ha⁻¹ of N before plantation, in a Spanish context [37] and rates of 120–150 kg ha⁻¹ y⁻¹ of N, 15–40 kg ha⁻¹ y⁻¹ of P, and 40 kg ha⁻¹ y⁻¹ of K for willow or poplar SRCs in Northern Europe [38].

The harvestable biomass nutrient average contents (kg ha^{-1}) in November 2021, considering a plant density of 5333 plants ha^{-1} and corresponding to the variables BioK, BioP, BioCu, BioZn, BioMg, and BioN, were obtained from chemical composition analysis and are given in Table 4. Nutrient removal in SRCs is generally greater than in conventional forestry due to the high density of the shoot population, characterized by small-sized stems and high bark volume [38]. Overall, these biomass variables showed an ascending tendency with slurry treatments, peaking with treatment T2. Thus, BioK, BioP, and BioCu ranged from 146 kg ha^{-1} (T0) to 313 kg ha^{-1} (T2), 23 kg ha^{-1} (T0) to 46 kg ha^{-1} (T2), and 0.3 kg ha^{-1} (T0 and T3) to 0.4 kg ha^{-1} (T1 and T2), respectively. BioZn, BioMg, and BioN ranged from 0.1 kg ha^{-1} (T0) to 1.0 kg ha^{-1} (T2), 10 kg ha^{-1} (T0) to 22 kg ha^{-1} (T2), and 218 kg ha^{-1} (T0) to 560 kg ha^{-1} (T2), respectively. These biomass contents were lower than those of the soil, both of which resulted from the cumulative effect of nine slurry treatments in 2019, 2020, and 2021 (Table 2). This reflects that, for each treatment, the plant biomass nutrient requirements were fully met by the slurry supply.

Table 4. Total biomass average contents of K_2O , P_2O_5 , Cu, Zn, Mg, and N (kg ha^{-1}) in 2021 for each treatment (kg ha^{-1}).

Treatment	BioK	BioP	BioCu	BioZn	BioMg	BioN
T0	146	23	0.3	0.1	10	218
T1	182	28	0.4	0.2	13	229
T2	313	46	0.4	1	22	560
T3	281	43	0.3	0.8	20	451

The net cumulative balances of soil and biomass nutrients in 2021, for each slurry treatment, given by the differences between the variables in Tables 2 and 4, are shown in Table 5. Positive net balances in Table 5, which were obtained from the differences between average soil and biomass nutrient contents in Tables 2 and 4, show that, as mentioned above, soil nutrient contents for all treatments were sufficient to cover plant biomass needs. All treatments showed positive differences, with K and Mg balances increasing from 502 kg ha^{-1} at T0 to 1068 kg ha^{-1} at T3, and from 343 kg ha^{-1} at T0 to 523 kg ha^{-1} at T3. Conversely, P showed a decreasing balance, from 187 kg ha^{-1} at T3 to 369 kg ha^{-1} at T0. The remaining nutrients showed oscillating tendencies, with N balance ranging from 3119 kg ha^{-1} (T2) to 4105 kg ha^{-1} (T3), Zn balance ranging from 1.9 kg ha^{-1} (T0) to 8.2 kg ha^{-1} (T3), and Cu balance ranging from 13.5 kg ha^{-1} (T0) to 28.5 kg ha^{-1} (T1).

Table 5. Net cumulative balances of soil and biomass nutrients for each treatment in 2021 (kg ha^{-1}), obtained from the differences between variables of soil nutrients in Table 2 and biomass nutrient contents in Table 4.

Treatment	K	P	Cu	Zn	Mg	N
T0	502	369	13.5	1.9	343	3733
T1	1048	272	28.5	7.3	376	3560
T2	1012	243	13.6	5	497	3119
T3	1068	187	15.1	8.2	523	4105

A site-specific evaluation of the saturation capacity of the soil–biomass vs. slurry system can be carried out through a proxy balance for each treatment. This balance between the cumulative demand for added OM and nutrients by the soil and biomass in 2021, and the cumulative nutrient and OM supply from the nine slurry applications, is

calculated as the difference between the cumulative demand and the cumulative supply. This balance for K, P, Cu, Zn, Mg, N, and OM, shown in Table 6, was thus derived by the sum of the biomass and soil nutrient and OM contents shown in Tables 2 and 4, subtracted from the nutrient slurry inputs corresponding to the nine slurry applications reported in Table 3. Overall, the positive values in Table 6 indicate that the inputs of seven nutrients and OM from the prescribed slurry treatments were matched by the absorption capacity of the soil–biomass system, suggesting that saturation of the soil and biomass by these components was not achieved.

Table 6. Net cumulative balances of nutrients and organic matter for soil and biomass vs. slurry nutrient inputs in 2021 for each treatment (kg ha^{-1}), resulting from the differences between the sum of nutrients and organic matter in soil and biomass in Tables 2 and 4 and slurry nutrients in Table 3.

Treatment	K	P	Cu	Zn	Mg	N	OM
T0	794	415	14	2.2	363	4170	63,571
T1	1015	136	28	4.4	294	3770	63,967
T2	848	−48	13	0.2	327	3742	71,359
T3	48	−493	13	−3.9	132	4013	40,821

Indeed, K, P, Cu, Zn, and Mg showed decreasing balances for the soil–plant system vs. slurry dosages. These balances ranged from 48 kg ha^{-1} (T3) to 1015 kg ha^{-1} (T1), $−493 \text{ kg ha}^{-1}$ (T3) to 415 kg ha^{-1} (T0), 13 kg ha^{-1} (T2 and T3) to 28 kg ha^{-1} (T1), $−3.9 \text{ kg ha}^{-1}$ (T3) to 4.4 kg ha^{-1} (T1), and 132 kg ha^{-1} (T3) to 363 kg ha^{-1} (T2). Thus, the balances of these nutrients showed the lowest amounts for the treatment T3, corresponding to the higher slurry dosage of 106.5 Mg ha^{-1} . N and OM balances showed oscillatory tendencies ranging from 4170 kg ha^{-1} (T0) to 3742 kg ha^{-1} (T2), and from $40,821 \text{ kg ha}^{-1}$ (T3) to $71,359 \text{ kg ha}^{-1}$ (T2). Treatment T2, corresponding to a slurry dosage of 53.2 Mg ha^{-1} , showed decreased balances of K, P, Cu, and Zn, which decreased compared with their values at T0 and T1, as well as a smaller N balance. Only treatment T1, corresponding to a slurry dose of 26.6 Mg ha^{-1} , ensured net soil–plant–slurry balances for the seven chemical components. In contrast, treatments T2 and T3 showed negative balances of $−48 \text{ kg ha}^{-1}$ and $−493 \text{ kg ha}^{-1}$ for P and of $−3.9 \text{ kg ha}^{-1}$ for Zn, for T3 only, as mentioned above.

After the first coppicing, we suggest that either a no-slurry application in the new rotation cycle, or at most a moderate application equivalent to treatment T1, should be adopted in the subsequent rotation cycle. This would allow the SRC system to reestablish a potential long-term, steady-state nutrient equilibrium condition through internal seasonal recycling processes such as litterfall decomposition and/or by retranslocation of nutrients from senescent to green leaves [37,39]. Indeed, one study by González et al. [40] in Central Spain, on a sandy-loam-textured soil without any fertilization, showed that annual litterfall in poplar SRC cultivations, with a density of $10,000 \text{ trees ha}^{-1}$, can be about $3.37 \text{ Mg ha}^{-1} \text{ y}^{-1}$, and nutrient retranslocation or nutrient resorption efficiency can be about 70%. Another study in France [41] strong nutrient recycling with a litterfall amount of $5 \text{ Mg ha}^{-1} \text{ y}^{-1}$, which caused a return of 60% to 80%, in each growing season, of the nutrients absorbed by plants through litter decomposition to the soil. This study corresponded to an SRC poplar system, with a density of $2000 \text{ trees ha}^{-1}$, a seven-year rotation cycle, and a fertilization of 65 kg P ha^{-1} before plantation and of 100 kgN ha^{-1} in the second year. In Central France, it was reported [42] that during the first rotation coppice of a poplar SRC with a density of $7272 \text{ trees ha}^{-1}$ without fertilization and under two rotation cycles of two years each, litterfall accounted for 26% of the aboveground biomass corresponding to $0.97 \text{ Mg ha}^{-1} \text{ y}^{-1}$, increasing to 36% and $3.06 \text{ Mg ha}^{-1} \text{ y}^{-1}$

during the second rotation. A study by Pérez et al. [43] on a poplar i214 clone SRC system in Spain, established at a high density of 25,000 trees ha⁻¹, reported an increasing annual tendency of nutrient input to soil through litter decomposition. This trend was associated with a rise in foliar biomass in the first rotation cycle, from 4.7 Mg ha⁻¹ y⁻¹ in the first year to 11.4 Mg ha⁻¹ y⁻¹ in the third year of that cycle. The same authors considered that, because the crop was established in a low-quality soil, fertilization of NPK with 48–40 and 75 kg ha⁻¹ should be carried out before planting for facilitation of poplar establishment. The same study, under a biomass productivity of 7.2 Mg ha⁻¹ y⁻¹, similar to that of this work (see below), reported that decomposing leaves during the first rotation cycle delivered amounts of N, P, and K of 180 kg ha⁻¹, 19 kg ha⁻¹, and 30 kg ha⁻¹ through litterfall. These values exceeded the aboveground biomass nutrient uptake estimates for the same period, which were 83 kg ha⁻¹, 8.7 kg ha⁻¹, and 29 kg ha⁻¹, respectively. This recycling potential supports minimizing fertilization in bioenergy cultivation under relatively fertile soil conditions, such as those in this work [20,40–43], which is also very relevant for site-specific planning of natural fertilization of poplar SRC cultivations with cattle slurry.

Proximate and ultimate analyses and high heating values (HHVs) of the biomass fuel quality are presented in Table 7. Overall, these values are within reported ranges for poplar biomass [7]. No notable difference in biomass components was detected among the four treatments, denoting that slurry application had no influence on the biomass fuel quality. In particular, the values of fixed carbon and HHVs, averaging 79% and 19.1 MJ kg⁻¹, respectively, fall within the usual range for this type of biomass, and are indicative of good fuel aptitude. These results for ultimate analyses and HHV are consistent with those reported by Paniagua et al. [44] for biomasses from experimental plots of four poplar SRC clones in Spain, which were subjected to annual soil amendments of 200 Mg ha⁻¹ of sludge from wastewater from the dairy industry, each delivering 117 kg ha⁻¹ of N in nitrate form over four years. The HHVs of biomass of the four clones with organic amendment ranged from 19.55 to 19.83 MJkg⁻¹, compared with HHVs ranging from 19.48 to 19.8 MJkg⁻¹ for biomass from poplar plots without treatment.

Table 7. Chemical proximate and ultimate analysis of poplar biomass in 2021 for each slurry treatment.

	Moisture (%)	Volatiles (%)	Ashes (%)	Fixed Carbon (%)	C (%)	H (%)	N (%)	O (%)	HHV (MJ kg ⁻¹)
T0	2.3	78.1	3.6	18.3	49.9	6.6	0.7	42.3	19.1
T1	2.5	79.1	3.2	17.6	49.7	6.6	0.8	42.6	18.4
T2	2.3	78.9	2.7	18.2	49.9	6.6	0.9	41.9	19.7
T3	2.3	78.9	3.3	17.6	49.7	6.4	0.7	42.5	19.1

3.2. Plant Biometry and Biomass Production

The values of plant height (TH) in m and of diameter at breast height (DBH) in cm of the main dominant shoots per sprout for the field plots representative of four slurry treatments in 2019, 2020, and 2021 are given in Table 8. The poplar plants grew to a maximum of 9.7 m for TH and 6.05 cm for DBH in 2021, and overall, there was a slightly increasing tendency in both biometric variables with the dosage of applied slurry, in combination with a stronger ascending annual tendency for each treatment. For example, while the average values of TH and DBH of treatments T0 and T3 in 2019 were 1.8 m and 1.9 m, and 0.8 cm and 0.8 cm, respectively, the corresponding average values of treatments T0 and T3 in 2021 were 7.7 m and 9.7 m, and 5.4 cm and 5.9 cm, respectively. The poplar plants' good performance in terms of TH and DBH can be attributed to their genetic traits. This reflects a high-level threshold in gains in key variables, such as wood productivity, disease resistance, or plant plasticity, about adaptation to different environments, which

are associated with advances in molecular biology and genetic manipulation aimed at optimizing the linkage between poplar genetics and physiology [2,45].

Table 8. Mean absolute values of poplar plant height (TH, m) and diameter at breast height (DBH, cm) of main dominant shoots in 2019, 2020, and 2021 for each slurry treatment.

	TH (m)		DBH (cm)	
	Mean	Std	Mean	Std
T0 2019	1.8	0.4	0.8	0.3
T1 2019	2.1	0.4	1	0.3
T2 2019	2.2	0.5	0.9	0.4
T3 2019	1.9	0.3	0.8	0.3
T0 2020	4.4	1.3	2.9	1.1
T1 2020	4.5	0.6	3.2	0.6
T2 2020	4.9	0.6	3.5	0.8
T3 2020	5.5	0.7	3.7	0.8
T0 2021	7.7	1.1	5.4	0.9
T1 2021	8.5	0.9	5.5	1.2
T2 2021	9.4	0.9	6	1
T3 2021	9.7	1.2	5.9	1.1

std—standard deviation.

From Table 8, the absolute mean growth increases in TH and DBH were obtained between 2019 and 2020 and 2020 and 2021 and presented, as mentioned above, as the variables Inc2019TH, Inc2019DBH, Inc2120TH, and Inc2120DBH in Table 9, respectively. Overall, the values of these variables showed ascendant tendencies, with average values of 2.4 m and 4.2 m for Inc2019TH and Inc2120TH, and 2.8 cm and 2.6 cm for Inc2019DBH and Inc2120DBH, respectively. Our results for plant TH and DBH, in Table 8, compare favorably with those reported in the study of Paniagua et al. [44], which examined experimental plots of poplar SRCs amended with organic sludge from dairy industry wastewater, where average plant height ranged from 1.2 m in the first year to 3.2 m in the fourth year. The corresponding values in untreated plots were 1 m and 2.5 m, respectively. For DBH, the average values for plants in treated and untreated plots ranged from 1.2 cm to 3.5 cm, and from 1 cm to 2.5 cm, respectively. A second case study of a poplar SRC fertilized with natural substrate was reported by Dimitriou and Aronsson [46], with plants grown in lysimeters irrigated with untreated municipal wastewater, wherein poplar plant height increased by between 20 cm and 110 cm during a 108-day initial growth period in response to application dosages of 30 kg P ha^{−1} and 315 kg N ha^{−1}. In this study, poplar plants also showed a strong capacity to retain N and P, with retention amounts exceeding 90%, and minimal risk of groundwater contamination. The good TH and DBH performance results, consistent with those of Dimitriou and Aronsson [46], suggest that the poplar plants were not stressed by the applied slurry dosages.

Table 9. Mean absolute increments in poplar plant height (m) and diameter at breast height (cm) of main dominant shoots between 2019 and 2020 (inc2019DBH and inc2019TH) and 2020 and 2021 (inc2120DBH and inc2120TH), mean relative increments (%) (definition in text) in poplar plant height and diameter at breast height between 2020 and 2019 (inrel2019TH and inrel2019DBH) and 2020 and 2021 (inrel2120TH and inrel2120DBH) and soil biomass productivity ($\text{kg ha}^{-1} \text{y}^{-1}$) (BioPr) for each slurry treatment.

Inc2019TH (m)	mean	std	Inrel2019TH (%)	mean	std
T0	2.1	0.9	T0	142.4	44.1
T1	2.2	0.5	T1	120.1	33.6
T2	2.5	0.7	T2	134.1	61.7
T3	2.8	0.7	T3	189.7	52.8
Inc2019DBH (cm)	mean	std	Inrel2019DBH (%)	mean	std
T0	2.5	1	T0	273.3	105.1
T1	2.4	0.5	T1	243.9	95.8
T2	2.7	0.8	T2	308.9	136.4
T3	3.5	0.6	T3	363.9	111.9
Inc2120TH (m)	mean	std	Inrel2120TH (%)	mean	std
T0	3.4	1.2	T0	87.1	39.5
T1	4.2	0.9	T1	94.2	10.7
T2	4.8	0.8	T2	105.9	21.9
T3	4.6	1.3	T3	93.8	34.7
Inc2120DBH (cm)	mean	std	Inrel2120DBH (%)	mean	std
T0	2.9	0.2	T0	132.0	54.2
T1	2.5	0.9	T1	86.3	36.2
T2	2.6	0.9	T2	74.9	28.4
T3	2.5	0.8	T3	73.2	21.3
BioPr ($\text{kg ha}^{-1} \text{y}^{-1}$)		mean	std		
T0		6165	1421		
T1		7730	1243		
T2		13,057	3126		
T3		11,842	2489		

Also shown in Table 9, for the four slurry treatments in 2021, are the biomass annual productivities (BioPr), which averaged $9700 \text{ kg ha}^{-1} \text{y}^{-1}$ and changed with an ascending tendency with slurry dosage, peaking with $13,057 \text{ kg ha}^{-1} \text{y}^{-1}$ under treatment T2.

Also, the biometric data for plant TH, DBH, and biomass productivity are consistent with typical values reported for the first rotation cycle of poplar SRCs [2,3,7,43,47,48] under developing root systems, which tend to increase in subsequent rotation cycles, when root systems are mature. In Table 9, the corresponding relative percent growth increases (Inrel2019TH, Inrel2019DBH, Inrel2120TH, and Inrel2120DBH) are also presented; these are defined, as mentioned above, by the dimensionless ratios between absolute increases in plant TH and DBH and the initial values of TH and DBH in the time interval that they refer to. The results of relative growth show that growth tendencies between 2020 and 2019, averaging 146% and 297%, for Inrel2019TH and Inrel2019DBH, respectively, are stronger than those between 2021 and 2020, averaging 95% and 91%, for Inrel2120TH and

Inc2120DBH, respectively. A statistical analysis giving evidence of the interplay between plant biometry, slurry, soil, and biomass chemical variables is shown below.

The annual N requirements of poplar SRCs, ranging from 93 to 122 kg ha⁻¹ y⁻¹ [37,40] or from 60 to 105 kg ha⁻¹ y⁻¹ [49], are considered high. The study of Wang et al. [36] showed that higher rates of carbon uptake, which are related to biomass allocation, are observed with minor N dosages of 115 kg ha⁻¹ y⁻¹ through three yearly applications, in comparison with higher dosages of 230 and 345 kg ha⁻¹ y⁻¹. From data on biomass chemical content and productivity, our study showed average values for N annual uptakes of 91, 95, 233, and 188 kg ha⁻¹ y⁻¹ for treatments T0, T1, T2, and T3, respectively. These values are within the ranges reported by Paris et al. [39], Wang et al. [36], and Heilman and Norby [49], indicating that the applied dosages of cattle slurry did not contribute to excessive N accumulation in biomass.

The amount of biomass weight productivity per unit of biomass nutrient content, commonly designated as nutrient use efficiency (NUE), is a useful indicator for evaluation of the productive impact of fertilization and for the selection of suitable clones for planting as well, with values varying considerably among clones and across rotation cycles [39]. Considering N, K, P, Ca, and Mg, biomass NUE in 2021 was shown as almost stationary across the four slurry treatments, with averages of 117, 304, 2680, 578, and 1760, which are in accordance with references for poplar SRCs [39,42]. In particular, as biomass production is strongly associated with N uptake, soil nitrogen use efficiency can be a relevant factor in the selection of planting material for SRC cultivations, given the relevance of N availability for the sustenance of plant growth and productivity [39,50].

3.3. Correlation and ANOVA Analysis of Results of Slurry, Soil, Biomass, and Plant Biometric Variables

The correlation matrix analysis for the evaluation of significant correlations between all 75 measured variables of four main classes, namely plant biometric slurry, soil, and biomass chemical variables, which considered the four slurry treatments as cases, was essential for analyzing the impact dynamics of slurry application on the soil and poplar plant system.

The categorical variable TREAT, representing the four slurry application treatments, displayed a total of 19 significant correlations which, besides significant correlations of 0.98 with 14 slurry variables, included a significant positive correlation of 0.98 with Inc2019DBH, of 0.99 with sOM19, and of −0.96, 0.97, and 0.96 with sP21, sFe21, and sMg21, respectively. Furthermore, each of the abovementioned 14 chemical components of the slurry displayed a total of 17 significant correlations, which, besides significant unitary correlations with the remaining 14 slurry variables, included significant correlations of 0.98 with Inc2019DBH, of 0.97 with sOM19, and of 0.99 with sK19. So, a set of six soil and plant biometric variables, including Inc2019DBH, sOM19, sP21, sFe21, sMg21, and sK19, were the most directly significantly impacted by the slurry treatments.

These directly impacted variables have, in turn, influenced others, which can be considered as indirectly impacted. Thus, Inc2019DBH showed significant correlations of 0.99 with sOM19 and of 0.97 with sMg21 and sK19. The variable sOM19 displayed a significant correlation of −0.97 with BioCu. The soil variables sK19 and sP21 also showed significant correlations of 0.96 with Inc2019TH and with Inc2120DBH, respectively. The soil variable sFe21 displayed two significant correlations of −0.97 with Inc2120DBH and of −0.99 with BioPb. The soil variable sMg21 showed significant correlations of 0.97 with BioPr and of −1 and 0.97 with BioCu and BioZn, respectively. Thus, sOM19, sMg21, and sK19 were soil variables also indirectly affected by the slurry treatments. The other set of eight indirectly impacted plant biometric and biomass variables included sInc2019TH, Inc2120DBH, Inc2120DBH, BioPr, BioPb, BioCu, and BioZn. A set of 14 soil, plant

biometric, and biomass variables directly or indirectly significantly impacted by the slurry treatments was therefore identified.

In short, concerning soil chemical composition, increases in slurry dosage to soil led to increases in sOM19, sK19, sFe21, and sMg21. Losses in sP21 also occurred. Considering plant biometrical variables, Inc2019DBH had the highest positive interaction with the slurry variables. The application of slurry to soil was reflected indirectly by a positive trend with BioPr via an increase in sMg21. The variable Inc2019TH was also indirectly and positively influenced through increases in sK19. On the other hand, IncDBH2120 and Increl2120DBH were indirectly and negatively influenced by the application of slurry into soil. The application of slurry to soil can also influence plant biochemistry dynamics. For example, Fe plays a key role in chlorophyll synthesis and cellular respiration [22]. The role of Mg in vital biochemical processes, for example, by being part of the chlorophyll molecule or by activation of enzymes in the Krebs cycle, is well established. Also, Mg, Fe, and Cd ions follow the same transport mechanisms within plants, with possible competitive interactions of these inorganic elements in both the apoplasm and symplasm regions of plants [19,22]. Potassium is a nutrient relevant to plant physiological processes such as nitrogen metabolism, carbohydrate metabolism in photosynthesis, and regulation of stomata closing, and is associated with over 50 enzymatic reactions in chlorophyll synthesis [19,22,51]. Biomass phytoremediation of excessive Pb or Cu would be negatively and indirectly impacted by increases in sOM19, in sFe21, and in sMg21, in contrast with Zn, whose incorporation by biomass would increase with slurry dosage due to an increase in sMg21. Within proper ranges, Zn can participate in auxin synthesis with direct influence in plant growth and biomass production, and copper can be an activator of enzymes catalyzing oxidation and reduction reactions. Elevated concentrations of these metals can result in deleterious competitive physiological interactions with other cations, plant growth inhibition, or toxicity symptoms [19,22].

The ANOVA modeling results, under a repeated measures general linear approach, allowed us to envision the significant differences between the least square means or contrasts of eight plant biometric and soil variables considered as dependent variables, which were directly or indirectly influenced by the independent categorical variable TREAT, which corresponds to the slurry treatment. The eight variables were Inc2019DBH, Inc2019TH, Inc2120DBH, Increl2120DBH, BioPr, sFe21, sP21, and sMg21. The results obtained are shown in Table 10. It can be noticed that for the variable Inc2019DBH, differences involving treatments T2 and T3 were significant, and that only the difference between T1 and T0 was not significant. This meant that overall, following the slurry treatments, a significant positive increase in the trunk diameter at breast height happened between 2020 and 2019, with an indirect impact on cumulative plant growth and biomass production in 2021. For Inc2019TH, only treatment T3 delivered significant direct increases in the differences between the mean of that variable and the corresponding means after treatments T0, T1, and T2. For the means of Inc2120DBH, only treatment T0 elicited significant differences with treatments T1, T2, and T3. The results for Increl2120DBH were the same as those for absolute enlargement, meaning that for this variable, whose impact, as mentioned above, was indirect and negative through sFe21, the negative impacts of treatments T1, T2, and T3 were marginal. The differences in mean values of BioPr were significant between treatment T0 and treatments T2 and T3, and between treatments T1 and T2, meaning that the indirect impact of slurry treatments on biomass productivity, through sMg21, was somewhat significant. This significance of dry biomass productivity with treatments agrees with the results of the abovementioned work of Ceotto et al. [20] on fertilization of a poplar SRC cultivation with cattle slurry in Italy, which reported that biomass yield was significantly influenced by slurry treatment. This statistical profile confirms that the use of fertilizers in intensively

managed bioenergy crops can be a determinant for achieving higher biomass yields, and in this context, the use of slurry can be an economical alternative to conventional fertilizers.

Table 10. ANOVA results for variables Inc2019DBH, Inc2019TH, Inc2120DBH, Inc2120DBH for the main dominant shoot and BioPr (definitions in text) for each slurry treatment in soil (* p -value < 0.05; ** otherwise).

Inc2019DBH (cm)	Slurry Treatment			
	T0	T1	T2	T3
T0		0.485 (**)	0.003 (*)	0.000009 (*)
T1	0.485 (**)		0.0058 (*)	0.000007 (*)
T2	0.003 (*)	0.0058 (*)		0.039 (*)
T3	0.000009 (*)	0.000007 (*)	0.039 (*)	
Inc2019TH (m)				
T0		0.38 (**)	0.45 (**)	0.0000 (*)
T1	0.38 (**)		0.051 (**)	0.0000 (*)
T2	0.45 (**)	0.051 (**)		0.0000 (*)
T3	0.0000 (*)	0.0000 (*)	0.0000 (*)	
Inc2120DBH (cm)				
T0		0.00000 (*)	0.00000 (*)	0.00000 (*)
T1	0.00000 (*)		0.52 (**)	0.63 (**)
T2	0.00000 (*)	0.53 (**)		0.80 (**)
T3	0.00000 (*)	0.63 (**)	0.80 (**)	
Incel2120DBH				
T0		0.045 (*)	0.014 (*)	0.012 (*)
T1	0.045 (*)		0.60 (**)	0.55 (**)
T2	0.014 (*)	0.60 (**)		0.94 (**)
T3	0.012 (*)	0.55 (**)	0.94 (**)	
BioPr (Mg ha ⁻¹ y ⁻¹)				
T0		0.70 (**)	0.019 (*)	0.03 (*)
T1	0.70 (**)		0.04 (*)	0.07 (**)
T2	0.019 (*)	0.04 (*)		0.76 (**)
T3	0.03 (*)	0.07 (**)	0.76 (**)	

The same ANOVA analysis for variables sFe21 and sP21 (Table 11) also delivered significant positive and negative differences in the mean values delivered by treatments T0 and T3, meaning that the remaining treatments exerted marginal influences on soil phosphorus and iron contents in 2021. For sMg21, only the mean differences resulting from treatments T0 and T1 were not significant, showing that the overall influence of the slurry treatments was more noticeable on that soil chemical component.

Table 11. ANOVA results for variables sFe21, sP21, and sMg21 (definitions in text) for each slurry treatment in soil (* p -value < 0.05; ** otherwise).

Slurry Treatment				
sFe21	T0	T1	T2	T3
T0		0.23 (**)	0.07 (**)	0.04 (*)
T1	0.23 (**)		0.48 (**)	0.30 (**)
T2	0.07 (**)	0.48 (**)		0.74 (**)
T3	0.04 (*)	0.30 (**)	0.74 (**)	
sP21				
T0		0.16 (**)	0.12 (**)	0.02 (*)
T1	0.16 (**)		0.86 (**)	0.28 (**)
T2	0.12 (**)	0.86 (**)		0.36 (**)
T3	0.02 (*)	0.28 (**)	0.36 (**)	
sMg21				
T0		0.21 (**)	0.0000 (*)	0.00001 (*)
T1	0.21 (**)		0.0000 (*)	0.0000 (*)
T2	0.0002 (*)	0.0000 (*)		0.0000 (*)
T3	0.00001 (*)	0.0000 (*)	0.0000 (*)	

Overall, the correlation and ANOVA analysis allowed us to conclude that a relatively moderate- to high-fertility soil, subjected to organic fertilization with cattle slurry, did not exert a relevant influence on plant biometry and biomass production. Indeed, only the increment in trunk DBH between 2020 and 2019 was directly and positively influenced by slurry treatments, with these initial increments being, of course, propagated in 2021. Slurry treatments, by directly impacting sP21 and sFe19, inclusively delivered indirect countering of the absolute and relative increments in trunk DBH between 2021 and 2020. Accumulated biomass production in 2021 was positively and indirectly influenced by the slurry treatments through an increase in sMg21 due to slurry treatments. This neutral response in poplar SRC cultivations has been mentioned for poplar [20] and willow [14] SRC plant biometry and biomass productivity, under conditions of relatively good soil fertility. These authors reported several examples of the absence of practical increases in biomass productivity under such soil conditions, especially during the first rotation cycle of the poplar coppices. This was likely related to factors such as nutrient cycling through litter decomposition, leaf fall, and fine root turnover, which reduce dependence on external nutrient inputs [36], or the immature and shallow root systems of young plants, which would lead to lower nutrient uptake from the soil and increase the shoot/root biomass ratio and which, by the end of the first rotation, would be deep enough to guarantee a higher supply of soil nutrients and a reduction in the aerial/root biomass ratio [48]. On the other hand, as the applied slurry provided relatively high N concentrations, the detrimental effects on the cultivations related to higher C/N ratios or N immobilization in soil would likely be mitigated [14,52–55].

3.4. Biometric Models

Biometric models were established for each slurry treatment in 2021 relating to (i) poplar tree biomass production with trunk DBH and tree TH, and (ii) poplar TH with trunk DBH.

For biomass production, the models were nonlinear, with the general form as follows:

$$\text{BioPr} = \beta_0(\beta_1\text{DBH}^{\beta_2})(\text{TH}^{\beta_3}) \quad (1)$$

established by least square estimation of the coefficients by numerical search.

For the relationship between poplar tree height and trunk diameter at breast height, the estimated models followed a linear least squares relationship as follows:

$$\text{TH} = \lambda_0 \text{DBH} + \lambda_1 \quad (2)$$

The coefficients for the nonlinear model are shown in Table 12.

Table 12. Coefficients for the nonlinear model relating plant biomass productivity with diameter at breast height.

Treatment	Coefficients				R ²
	β_0	β_1	β_2	β_3	
T0	0.055	0.061	4.31	−0.34	0.89
T1	0.051	0.060	1.18	2.30	0.90
T2	0.21	0.22	1.11	1.21	0.98
T3	0.22	0.20	1.72	0.72	0.98

The linear models relating poplar TH and trunk DBH, corresponding to the general form in (2), were the following:

$$y = 2.25x - 5.23 \quad R^2 = 0.63$$

$$y = 1.73x - 2.62 \quad R^2 = 0.89$$

$$y = 0.65x + 5.44 \quad R^2 = 0.60$$

$$y = 0.92x + 4.31 \quad R^2 = 0.72$$

3.5. Principal Component (PCA) and Factorial Analysis (FA)

Principal component analysis (PCA) of the set of 14 plant biometric, soil, slurry, and biomass variables influenced significantly (directly or indirectly) by the slurry treatment in the plantation showed that two main PCs (Table 13) could explain 86% and 7% of the total variability of this set. The absolute values of correlations of the variables with the first PC were higher than 0.7, with 11 variables with correlations higher than 0.9, showing that this PC is a good representative of the 14-variable set. A factorial analysis with varimax rotation with two factors (Table 14 and Figure 2) showed that the variables TREAT, Inc2019DBH, Inc2019TH, sMO19, sMg21, sK19, BioPr, BioCu, and BioZn display higher loadings of Factor 1, while variables sP21, sFe21, Inc2120DBH, Inc2120DBH, and BioPb, exhibit higher loadings of Factor 2.

The loading plot of each variable in Graph 1 reflects the correlation pattern described above, and thereby the good representativeness of the 14-variable set for the characterization of the cultivation system. In Figure 2, two groups of variables are shown: sP21, BioCu, Inc2120DBH, Inc2120DBH, and BioPb on one hand, and TREAT, Inc2019DBH, sMO19, sK19, sFe21, sMg21, Inc2019TH, BioPr, and BioZn on the other hand. These two groups of variables clearly reflect the abovementioned correlation patterns, caused by the application of slurry to soil. In this set, the variable TREAT reflects the impact of the slurry treatments, the variables sK19, sP21, sFe21, and sMg21 can be considered as essential

for the soil–plant interactions, and the variables BioZn, BioCu, and BioPb can be envisaged as participating, with negative or positive influences, in plant phytoremediation and/or biochemistry processes. From the information mentioned above, the fertilization of the poplar SRC cultivation with bovine slurry led to a slight response in biomass productivity, along with a strong capacity of the soil–plant system to absorb the nutrients, in parallel with values of nutrient use efficiency for N, K, P, Ca and Mg, which were typical of these cultivations and remain relatively stationary across slurry dosages.

Table 13. Principal component analysis for a set of 14 plant biometric, slurry, soil, and biomass variables.

Variables	Principal Component 1	Principal Component 2	Principal Component 3
TREAT	−1.00	−0.01	0.07
Inc2019DBH	−0.98	−0.20	0.06
sMO19	−1.00	−0.06	−0.02
sP21	0.95	−0.25	−0.21
sFe21	−0.97	0.22	−0.05
sMg21	−0.98	−0.12	−0.19
sK19	−0.92	−0.24	0.31
BioCu	0.95	0.17	0.26
Inc2019TH	−0.79	−0.46	0.41
Inc2120DBH	0.81	−0.49	−0.32
Increl2120DBH	0.89	−0.45	0.08
BioPb	0.94	−0.31	0.16
BioPr	−0.92	−0.10	−0.38
BioZn	−0.90	−0.23	−0.39

Table 14. Factorial analysis with varimax rotation for a set of 14 plant biometric, slurry, soil, and biomass variables.

Variables	Factor 1	Factor 2
TREAT	0.74	0.67
Inc2019DBH	0.86	0.51
sMO19	0.78	0.63
sP21	−0.53	−0.82
sFe21	0.57	0.82
sMg21	0.80	0.57
sK19	0.84	0.44
BioCu	−0.82	−0.52
Inc2019TH	0.89	0.19
Inc2120DBH	−0.27	−0.91
Increl2120DBH	−0.36	−0.93
BioPb	−0.49	−0.86
BioPr	0.75	0.55
BioZn	0.81	0.44

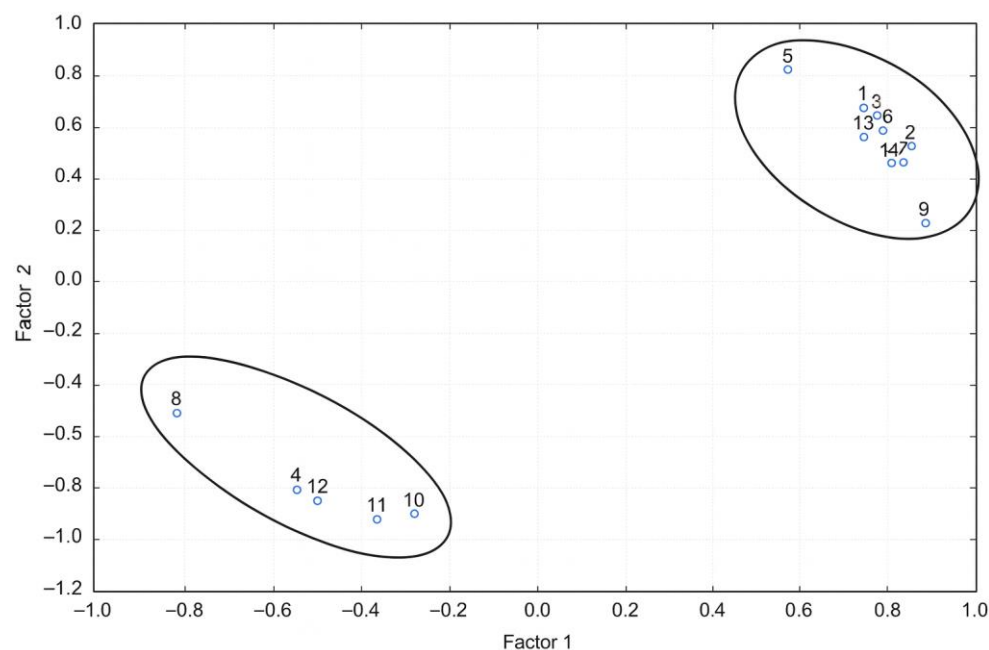


Figure 2. Loadings of two factors obtained with factorial analysis with varimax rotation from the set of plant biometric, slurry, soil, and biomass variables referred to in the legend. 1—TREAT; 2—Inc2019DBH; 3—sMO19; 4—sP21; 5—sFe21; 6—sMg21; 7—sK19; 8—BioCu; 9—Inc2019TH; 10—Inc2120DBH; 11—Incel2120DBH; 12—BioPb; 13—BioPr; 14—BioZn.

3.6. Practical Implications of the Present Study

In Portugal, bovine slurry could be used as a natural fertilizer for a proposed potential total area of poplar SRCs of about 61 k ha [56]. Organic fertilization with bovine slurry will enhance the financial feasibility of poplar SRC cultivations by reducing reliance on chemical fertilizer and the corresponding costs, thereby allowing for significant increases in gross margins [57]. The energy balances of poplar SRC cultivations are elucidative of their relevance as decarbonizing drivers. For example, SRC cultivations were shown to deliver as much as 86 times more energy per unit of fossil energy input in comparison with fossil coal, and the energy ratios of poplar SRC cultivations range between 13 and 79 from cradle to farm gate, with biomass yield being a key factor contributing to these ratios [58]. In this study, the energy output from biomass, assuming an average biomass productivity by the poplar SRC cultivation of $9.7 \text{ Mg ha}^{-1} \text{ y}^{-1}$, was about $184.3 \text{ GJ ha}^{-1} \text{ y}^{-1}$, corresponding to a sequestration of $17.8 \text{ Mg CO}_2 \text{ ha}^{-1} \text{ y}^{-1}$, and potentially avoiding about $14 \text{ Mg CO}_2 \text{ ha}^{-1} \text{ y}^{-1}$ of fossil fuel emissions. The highest cattle slurry dosage for application in soil that we considered was $320 \text{ Mg ha}^{-1} \text{ y}^{-1}$. For this application, about 28.5 GJ y^{-1} of energy for mechanical operations with slurry is required, with about 62.7 MJ y^{-1} , 6.2 GJ y^{-1} , 1.9 GJ y^{-1} , and 0.1 GJ y^{-1} corresponding to pumping, agitation, transport for 20 km, and soil application, respectively. The fossil fuel carbon emissions corresponding to such mechanical operations are about $3.1 \text{ Mg CO}_2 \text{ y}^{-1}$, with about 4.7 kg y^{-1} , $1.1 \text{ Mg CO}_2 \text{ y}^{-1}$, $1.9 \text{ Mg CO}_2 \text{ y}^{-1}$, and 0.1 corresponding to pumping, agitation, transport for 20 km, and soil application, respectively [59–62]. Both the energy and the carbon emissions, are of smaller orders of magnitude than those concerning the biomass output of the cultivation. Intensive poplar SRC cultivations require energy inputs between 3 and $16 \text{ GJ ha}^{-1} \text{ y}^{-1}$ related to direct energy inputs such as diesel or electricity and to indirect energy inputs including fertilizers [58,63]. Fertilization energy inputs range from 10% to 64% of the total, and fertilizing production is energy-intensive with requirements as high as 10 GJ for producing 200 kg of nitrogen fertilizer [50,63]. Production of the T1 slurry application of $79.8 \text{ Mg ha}^{-1} \text{ y}^{-1}$ requires about 4.4 cows y^{-1} , considering an average daily

production of 50 L per cow. The corresponding CO₂-eq emissions, considering an emission of 120 kg cow⁻¹ y⁻¹, are about 1.45 Mg ha⁻¹ y⁻¹ in stoichiometric terms and about 14.36 Mg ha⁻¹ y⁻¹ if a Global Warming Potential in 100 years (GWP100) of 27.2 is considered [63,64]. So, for treatment T1, the carbon budget of poplar cultivation, with an average carbon sequestration of 17.8 Mg CO₂ ha⁻¹ y⁻¹, combined with the applied slurry, is overall neutral both in stoichiometric and in GWP100 terms. For treatments T2 and T3, with slurry dosages higher by twofold and threefold orders of magnitude, the corresponding CO₂-eq emissions in GWP100 terms are about 28.7 Mg ha⁻¹ y⁻¹ and 58.75 Mg ha⁻¹ y⁻¹, surpassing the average carbon sequestration by the poplar cultivation. Thus, with treatments T2 and T3, despite the fact that the theoretical stoichiometric carbon budget will remain overall neutral, the impact of a high GWP20 prevails, with the whole system working as a carbon source with negative climatic impact. Treatment T1 corresponds thereby to a threshold for keeping nutrient equilibrium and an absence of negative impacts related to atmospheric warming. With treatments T2 and T3, the use of slurry as a natural fertilizer in poplar cultivation only partially mitigates the effect of methane emissions from cattle, which would exist anyway, and thus additional measures may be needed—such as the addition of biochar to soil delivering carbon sequestration in the long-term, grazing that increases SOC, or dietary changes to reduce enteric CH₄. This limitation would be reduced with an increase of about 20% in biomass productivity in subsequent rotation cycles of SRC cultivation with trees with mature root systems [2,3,48]. For the effects of ecological profitability, under the threat that global climatic changes pose to biological species, biodiversity conservation should also be considered in the planning of poplar SRC cultivations [65]. Microbiota and macrobiota associated with these cultivations include fungi, bacteria and protozoa, annelids, arthropods, arachnids, mites, birds, and insects [10,65]. In terms of biodiversity, as with management, SRCs stand between agriculture and classic forestry, and the main influence of slurry application on α - and β -diversity is mainly indirect due to its impact on key cultivations factors [65]. Slurry application is also ecologically relevant, as it represents a form of community coalescence; here, distinct microbial communities come into contact [66]. However, key questions about the persistence of slurry-derived microbiota and their interactions with resident communities underscore the need for future studies on how repeated slurry inputs may alter native soil microbial functions over time. Rapid tree growth is also a determinant of positive effects in the ecological habitability of SRCs for animals, such as breeding birds, which depend heavily on vertical structures. Also, low pesticide and fertilizer requirements are beneficial to invertebrates such as butterflies and moths, earthworms, springtail species (*Collembola*), arachnids, and arthropods [10,65].

The biodiversity of SRC cultivations is, of course, influenced by the surrounding landscape, and installing SRCs has a positive effect on isolated landscapes, like the one in this study. Also, more edges in plot shapes are also useful because species richness tends to decrease from the edge towards the interior, possibly because plant seeds can colonize edges more easily through wind or because beyond edges, vegetation becomes more uniform and compact, and hence more difficult to colonize for other plant species. In a minimal SRC area of 4000 m² or larger, like that in this study, coppicing is a major disruption to the canopy due to the removal of the whole aboveground biomass and should be carried out in a way to deliver temporal and spatial mosaics of canopy structures able to maintain niches of biodiversity. The balance between maximum environmental effects and maximum attained biomass production from SRCs is a big challenge to be dealt with by the diverse stakeholders involved in SRC cultivations, such as researchers, farmers, and decision-makers [17]. The inclusion of cattle slurry in the lifecycle of poplar SRC cultivations can thereby add direct and indirect contributions to the reduction in emissions of greenhouse gases, while enabling a potential increase in biomass productivity due to nutrient inputs. This kind of organic fertilization, especially under treatment T1, would also promote circularity in agroecosystems, insofar as cattle slurry from local meat

and milk production can be incorporated into and retrofitted for biomass and bioenergy cultivations, while maintaining long-term steady functioning of these cultivations in terms of nutrient equilibrium, biomass production, and neutral carbon balance. In this context, we suppose, with this site-specific analysis, that bovine slurry with controlled dosages can be an acceptable choice to fertilize poplar SRC cultivations, and that the whole range of economic and environmental principles involved can contribute to decentralized boosting of rural economies.

4. Conclusions

This work showed that, in a moderately fertile site with a good potential for the installation of poplar SRC cultivation, fertilization with cattle slurry is a viable option for the valorization of this feedstock from intensive dairy cattle breeding. A steady state of the agroecosystem, evidenced by the mass balances of K, P, Cu, Zn, Mg, and N and organic matter in the whole soil, plant, and slurry system, was achieved by the end of the first rotation cycle with the application of four dosages of 0, 26.6, 53.2, and 106.5 Mg ha⁻¹, designated as treatments T0, T1, T2, and T3, three times per year during three years. Mass balances of the whole soil–biomass–slurry system showed that, overall, nutrient saturation of the whole soil–biomass–slurry system was not achieved. The cumulative supply of nutrients was higher than cumulative demand, except for T2 and T3 with P and T3 with Zn, supporting no slurry application, or at most a moderate application equivalent to T1 in the second rotation cycle, so that a nutrient equilibrium status can be achieved through internal seasonal recycling mechanisms. Plant growth variables related to total height (TH) and diameter at breast height (DBH) were positively influenced by the slurry treatments, mainly in the transition between 2019 and 2020, with average relative increments of TH and DBH ranging between 120 % and 189 % and 273 % and 364 %, respectively. The biomass productivity was only indirectly affected by slurry dosages, with average values varying from 6.1 to 11.8 Mg ha⁻¹ y⁻¹ and peaking with treatment T2, within a range typical of a first rotation cycle. The biomass fuel quality was not affected. The environmental and financial feasibilities of natural fertilization with slurry are enhanced because the energy spent in the production of chemical fertilizers, along with the correspondent costs and fossil fuel carbon emissions, are eliminated. Only in treatment T1 were the methane cattle CO₂-eq emissions, overall equilibrated by the carbon sequestration of the poplar cultivation, assuring an absence of climatic-warming impacts. Treatment T1 in the agroecosystem thereby provided an environmental threshold, regarding long-term nutrient equilibrium, bioenergy production, and absence of negative impacts due to atmospheric warming. Anyway, a main conclusion was that the integration of cattle slurry in this biomass cluster could result in circular gains for cattle and bioenergy producers with relevant decentralized contributions for boosting the social-economic dynamics of rural communities.

Author Contributions: Conceptualization, A.R., S.P., A.A.G., T.F., P.C. and R.M.; methodology, A.R., S.P., A.A.G., J.L., T.F., A.C.-B., P.C., H.M. and R.M.; software, A.R. and A.B.G.; validation, A.R., S.P., A.B.G. and R.M.; formal analysis, A.R. and A.B.G.; investigation, A.R., S.P., A.A.G., J.L., T.F., A.C.-B., P.C., H.M. and R.M.; resources, A.R. and R.M.; data curation, A.R., S.P., J.L., A.B.G., T.F., A.C.-B. and R.M.; writing—original draft preparation, A.R.; writing—review and editing, A.R., S.P., A.B.G. and R.M.; visualization, A.R., S.P., A.B.G., H.M. and R.M.; supervision, A.R., A.A.G., T.F., A.C.-B., P.C. and R.M.; project administration, A.R. and R.M.; funding acquisition, A.R. and R.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the PDR 2020—Programa de Desenvolvimento Rural 2014—2020, Project GO-BioChorume, Ref. PDR2020-101-032093. This work is supported by National Funds by FCT—Portuguese Foundation for Science and Technology under the projects LAETA Programmatic Funding (DOI: 10.54499/UIDP/50022/2020), CERIS Research Center (DOI: 10.54499/UIDB/04625/2020), UID/04033/2025: Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Institute for innovation, capacity building and sustainability of agri-food production (DOI: 10.54499/LA/P/0126/2020), and CBQF: Centre for Biotechnology and Fine Chemistry—UIDB/50016/2020. Sofia I.A. Pereira thanks FCT for the Assistant Researcher contract—2023. 15056.TENURE.047—through the FCT-TENURE Program funded by the Recovery and Resilience Plan (PRR).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: We thank Aveleda S.A. (Portugal) and Rita Guedes for the logistical support and coordination essential to develop Project GO-BioChorume and this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Oliveira, N.; Rodríguez-Soalleiro, R.; Hernández, M.J.; Cañellas, I.; Sixto, H.; Pérez-Cruzado, C. Improving biomass estimation in a *Populus* short rotation coppice plantation. *For. Ecol. Manag.* **2017**, *391*, 194–206. [CrossRef]
- Oliveira, N.; Pérez-Cruzado, C.; Cañellas, I.; Rodríguez-Soalleiro, R.; Sixto, H. Poplar Short Rotation Coppice Plantations under Mediterranean Conditions: The Case of Spain. *Forests* **2020**, *11*, 1352. [CrossRef]
- Rodrigues, A.; Costa, M.; Nunes, L. Short rotation woody coppices for biomass production: An integrated analysis of the potential as an energy alternative. *Curr. Sustain. Renew. Energy Rep.* **2021**, *8*, 70–89. [CrossRef]
- Šrámek, M.; Weger, J.; Bubeník, J.; Matoušková, M.; Lengálová, K.; Matula, R. Effective woody biomass estimation in poplar short-rotation coppices—*Populus nigra* × *P. maximowiczii*. *iForest—Biogeosciences For.* **2023**, *16*, 202–209. [CrossRef]
- Capuana, M. Heavy metals and woody plants—Biotechnologies for phytoremediation. *iForest—Biogeosciences For.* **2011**, *4*, 7–15. [CrossRef]
- Kafle, A.; Timilsina, A.; Gautam, A.; Adhikari, K.; Bhattarai, A.; Aryal, N. Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. *Env. Adv.* **2022**, *8*, 100203. [CrossRef]
- Stochlova, P.; Novotná, K.; Costa, M.; Rodrigues, A. Biomass production of poplar short rotation coppice over five and six rotations and its aptitude as a fuel. *Biomass Bioenergy* **2019**, *122*, 183–192. [CrossRef]
- Rockwood, D.L.; Naidu, C.V.; Carter, D.R.; Rahmani, M.; Spriggs, T.A.; Lin, C.; Alker, G.R.; Isebrands, J.G.; Segrest, S.A. Short-rotation woody crops and phytoremediation: Opportunities for agroforestry? *Agrofor. Syst.* **2004**, *61*, 51–63. [CrossRef]
- Padoan, E.; Passarella, I.; Prati, M.; Bergante, S.; Facciotto, G.; Ajmone-Marsan, F. The Suitability of Short Rotation Coppice Crops for Phytoremediation of Urban Soils. *Appl. Sci.* **2020**, *10*, 307. [CrossRef]
- Dimitriou, I.; Baum, C.; Sarah Baum, S.; Busch, G.; Schulz, U.; Köhn, J.; Lamersdorf, N.; Leinweber, P.; Aronsson, P.; Weih, M.; et al. Quantifying Environmental Effects of Short Rotation Coppice (SRC) on Biodiversity, Soil and Water. *IEA Bioenergy Report: Task 43: 2011:01, 34 pps; IEA Bioenergy Secretariat Website*. Available online: www.ieabioenergy.com (accessed on 23 July 2025).
- Forbes, E.G.A.; Johnston, C.R.; Archer, J.E.; McCracken, A.R. SRC willow as a bioremediation medium for a dairy farm effluent with high pollution potential. *Biomass Bioenergy* **2017**, *105*, 174–189. [CrossRef]
- Provolo, G.; Manuli, G.; Finzi, A.; Lucchini, G.; Riva, E.; Sacchi, G.A. Effect of Pig and Cattle Slurry Application on Heavy Metal Composition of Maize Grown on Different Soils. *Sustainability* **2018**, *10*, 2684. [CrossRef]
- Menino, R.; Moreira, H.; Castelo-Branco, A.; Gomes, A.A.; Rodrigues, A.; Castro, P.M.L.; Cunha, J.; Guedes, R.; Pereira, S.I.A. Fertilizer potential of slurry from intensive dairy cattle farms in intensive production forestry systems. *Int. J. Hydrol.* **2022**, *6*, 44–47. [CrossRef]
- Quaye, A.K.; Volk, T.A.; Hafner, S.; Leopold, D.J.; Schirmer, C. Impacts of paper sludge and manure on soil and biomass production of willow. *Biomass Bioenergy* **2011**, *35*, 2796–2806. [CrossRef]
- Quaye, A.K.; Volk, T.A. Biomass production and soil nutrients in organic and inorganic fertilized willow biomass production systems. *Biomass Bioenergy* **2013**, *57*, 113–125. [CrossRef]
- Quaye, A.K.; Volk, T.A.; Schoenau, J. Seasonal Dynamics of N, P, and K in an Organic and Inorganic Fertilized Willow Biomass System. *Appl. Environ. Soil Sci.* **2015**, *2015*, 471248. [CrossRef]

17. Dimitriou, I.; Baum, C.; Baum, S.; Busch, G.; Schulz, U.; Köhn, J.; Lamersdorf, N.; Leinweber, P.; Aronsson, P.; Weih, M.; et al. The impact of Short Rotation Coppice (SRC) cultivation on the environment. *Landbauforschung* **2009**, *59*, 159–162.
18. Senbayram, M.; Chen, R.; Budai, A.; Bakken, L.; Dittert, K. N₂O emission and the N₂O/(N₂O+N₂) product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. *Agric. Ecosyst. Environ.* **2012**, *147*, 4–12. [[CrossRef](#)]
19. Pajević, S.; Borišev, M.; Nikolić, N.; Arsenov, D.D.; Orlović, S.; Župunski, M. Phytoextraction of Heavy Metals by Fast-Growing Trees: A Review. In *Phytoremediation*; Ansari, A., Gill, S., Gill, R., Lanza, G., Newman, L., Eds.; Springer: Cham, Switzerland, 2016.
20. Ceotto, E.; Castelli, F.; Moschella, A.; Diozzi, M.; Di Candilo, M. Poplar short rotation coppice is not a first-choice crop for cattle slurry fertilization: Biomass yield and nitrogen-use efficiency. *Ind. Crops Prod.* **2016**, *85*, 167–173. [[CrossRef](#)]
21. Robertson, G.P.; Groffman, P.M. 13—Nitrogen Transformations. In *Soil Microbiology, Ecology and Biochemistry*, 3rd ed.; Paul, E.A., Ed.; Academic Press: Oxford, UK, 2007; pp. 341–364. ISBN 9780125468077. [[CrossRef](#)]
22. Chatzistathis, T.; Therios, I. How Soil Nutrient Availability Influences Plant Biomass and How Biomass Stimulation Alleviates Heavy Metal Toxicity in Soils: The Cases of Nutrient Use Efficient Genotypes and Phytoremediators, Respectively. In *Biomass Now—Cultivation and Utilization*; Matovic, M., Ed.; IntechOpen: London, UK, 2013; ISBN 978-953-51-1106-1. [[CrossRef](#)]
23. Khan, I.M.; Alam, S.; Anum, S.; Niazi, K.N. Phytoremediation of Agricultural Pollutants. In *Phytoremediation. Concepts and Strategies in Plant Sciences*; Shmaefsky, B., Ed.; Springer: Cham, Switzerland, 2020. [[CrossRef](#)]
24. Khan, M.; Shaheen, S.; Ali, S.; Yi, Z.; Cheng, L.; Khan, D.M.; Azam, M.; Rizwan, M.; Afzal, M.; Irum, G.; et al. In Situ Phytoremediation of Metals. In *Phytoremediation. Concepts and Strategies in Plant Sciences*; Shmaefsky, B., Ed.; Springer: Cham, Switzerland, 2020. [[CrossRef](#)]
25. Khan, A.G. In Situ Phytoremediation of Uranium Contaminated Soils. In *Phytoremediation. Concepts and Strategies in Plant Sciences*; Shmaefsky, B., Ed.; Springer: Cham, Switzerland, 2020. [[CrossRef](#)]
26. Via, S.M. Phytoremediation of Explosives. In *Phytoremediation. Concepts and Strategies in Plant Sciences*; Shmaefsky, B., Ed.; Springer: Cham, Switzerland, 2020. [[CrossRef](#)]
27. Wijekoon, W.; Priyashantha, H.; Gajanayake, P.; Manage, P.; Liyanage, C.; Jayarathna, S.; Kumarasinghe, U. Review and Prospects of Phytoremediation: Harnessing Biofuel-Producing Plants for Environmental Remediation. *Sustainability* **2025**, *17*, 822. [[CrossRef](#)]
28. Bremner, J.M.; Mulvaney, C.S. Total Nitrogen. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological 14 Properties*, 2nd ed.; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; American Society of Agronomy, Inc.: Madison, WI, USA, 1982; pp. 595–624.
29. Walkley, A. A critical examination of a rapid method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.* **1947**, *63*, 251–263. [[CrossRef](#)]
30. Balbino, L.R. O método de Egnér-Riehm na determinação do fósforo e do potássio “assimiláveis” em solos de Portugal. *Rev. Agronómica* **1968**, *51*, 46–56. (In Portuguese)
31. Ceotto, E.; Castelli, F.; Moschella, A.; Diozzi, M.; Di Candilo, M. It is not worthwhile to fertilize sweet sorghum (*Sorghum bicolor* L. Moench) with cattle slurry: Productivity and nitrogen-use efficiency. *Ind. Crops Prod.* **2014**, *62*, 380–386. [[CrossRef](#)]
32. Ketterings, Q.; Klausner, S.; Czymmek, K. *Nitrogen Guidelines for Field Crops in New York. Second Release*; Department of Crop and Soil Extension Series E03-16, Cornell University: Ithaca, NY, USA, 2003; 70p.
33. Ceotto, E.; Castelli, F.; Moschella, A.; Diozzi, M.; Di Candilo, M. Cattle Slurry Fertilization to Giant Reed (*Arundo donax* L.): Biomass Yield and Nitrogen Use Efficiency. *Bioenergy Res.* **2015**, *8*, 1252–1262. [[CrossRef](#)]
34. Adegbedi, H.G.; Volk, T.A.; White, E.H.; Abrahamson, L.P.; Briggs, R.D.; Bickelhaupt, D.H. Biomass and nutrient removal by willow clones in experimental bioenergy plantations in New York State. *Biomass Bioenergy* **2001**, *20*, 399–411. [[CrossRef](#)]
35. Di Matteo, G.; Sperandio, G.; Verani, S. Field performance of poplar for bioenergy in southern Europe after two coppicing rotations: Effects of clone and planting density. *iForest* **2012**, *5*, 224–229. [[CrossRef](#)]
36. Wang, Y.; Xi, B.; Bloomberg, M.; Moltchanov, E.; Li, G.; Jia, L. Response of diameter growth, biomass allocation and N uptake to N fertigation in a triploid *Populus tomentosa* plantation in the North China Plain: Ontogenetic shift does not exclude plasticity. *Eur. J. For. Res.* **2015**, *134*, 889–898. [[CrossRef](#)]
37. Blanco, H.; Garasa, M.; Ciria, M.; García, J.; de Viñas, I. Manual de cultivo de *Populus* spp. para la producción de biomasa con fines energéticos. *Monogr. INIA Ser. Florest.* **2010**, *21*, 60. (In Spanish)
38. Dimitriou, R.; Rutz, D. Sustainable Short Rotation Coppice. In *A Handbook*; WIP Renewable Energies: Munich, Germany, 2015; 103p, ISBN 978-3-936338-36-2.
39. Paris, P.; Mareschi, L.; Sabatti, M.; Tosi, L.; Scarascia-Mugnozza, G. Nitrogen removal and its determinants in hybrid *Populus* clones for bioenergy plantations after two biennial rotations in two temperate sites in northern Italy. *iForest* **2015**, *8*, 657–665. [[CrossRef](#)]
40. González, I.; Sixto, H.; Rodríguez-Soalleiro, R.; Oliveira, N. Nutrient Contribution of Litterfall in a Short Rotation Plantation of Pure or Mixed Plots of *Populus alba* L. and *Robinia pseudoacacia* L. *Forests* **2020**, *11*, 1133. [[CrossRef](#)]

41. Berthelot, A.; Ranger, J.; Gelhaye, D. Nutrient uptake and immobilization in a short-rotation coppice stand of hybrid poplars in north-west France. *For. Ecol. Manag.* **2000**, *128*, 167–179. [\[CrossRef\]](#)
42. Guénou, R.; Bastien, J.C.; Thiébeau, P.; Bodineau, G.; Bertrand, I. Carbon and nutrient dynamics in short-rotation coppice of poplar and willow in a converted marginal land, a case study in central France. *Nutr. Cycl. Agroecosystems* **2016**, *106*, 293–309. [\[CrossRef\]](#)
43. Pérez, P.; Barro, R.; Pérez, J.; Fernández, M.J.; Moyano, A.; Ciria, P. Nutrient Release through Litterfall in Short Rotation Poplar Crops in Mediterranean Marginal Land. *Forests* **2021**, *12*, 1185. [\[CrossRef\]](#)
44. Paniagua, S.; Escudero, C.; Escapa, R.; Coimbra, M.; Calvo, L. Effect of waste organic amendments on *Populus* sp biomass production and thermal characteristics. *Renew. Energy* **2016**, *94*, 166–174. [\[CrossRef\]](#)
45. Ceulemans, R.; Deraedt, W. Production physiology and growth potential of poplars under short-rotation forestry culture. *For. Ecol. Manag.* **1999**, *121*, 9–23. [\[CrossRef\]](#)
46. Dimitriou, I.; Aronsson, P. Wastewater and sewage sludge application to willows and poplars grown in lysimeters-Plant response and treatment efficiency. *Biomass Bioenergy* **2011**, *35*, 161–170. [\[CrossRef\]](#)
47. Liberloo, M.; Calfapietra, C.; Lukac, M.; Godbold, D.; Luo, Z.-B.; Pol, A.; Hoosbeek, M.; Kull, O.; Marek, M.; Raines, C.; et al. Woody biomass production during the second rotation of a bio-energy *Populus* plantation increases in a future high CO₂ world. *Glob. Change Biol.* **2006**, *12*, 1094–1106. [\[CrossRef\]](#)
48. Rodrigues, A.; Pita, G.; Mateus, J.; Kurz-Besson, C.; Casquilho, M.; Cerasoli, S.; Gomes, A.; Pereira, J. Eight years of continuous carbon fluxes measurements in a Portuguese eucalypt. *Agric. For. Meteorol.* **2011**, *151*, 493–507. [\[CrossRef\]](#)
49. Heilman, P.; Norby, R. Nutrient Cycling and Fertility Management in Temperate Short Rotation Forest Systems. *Biomass Bioenergy* **1998**, *14*, 361–370. [\[CrossRef\]](#)
50. van Veen, J.; Breteler, H.; Olie, J.; Frissel, M.J. Nitrogen and energy balance of a short-rotation poplar forest system. *Neth. J. Agric. Sci.* **1981**, *29*, 163–172. [\[CrossRef\]](#)
51. Rodrigues, A.; Vanbeveren, S.; Costa, M.; Ceulemans, R. Relationship between soil chemical composition and potential fuel quality of biomass from poplar short rotation coppices in Portugal and Belgium. *Biomass Bioenergy* **2017**, *105*, 66–72. [\[CrossRef\]](#)
52. Vasconcelos, E.; Cabral, F. Use and environmental implications of pulp-mill sludge as an organic fertilizer. *Environ. Pollut.* **1993**, *80*, 159–162. [\[CrossRef\]](#)
53. Lee, C.-Y.; Kim, C.-H.; Park, H.-H.; Park, M.-S.; Lee, C.-H.; Park, J.-H. Potential Use of Paper Mill Sludge in Improving Soil Quality for Plant Growth. *Appl. Sci.* **2023**, *13*, 8723. [\[CrossRef\]](#)
54. Bellamy, K.; Chong, C.; Cline, R. Paper sludge utilization in agriculture and container nursery culture. *J. Environ. Qual.* **1995**, *24*, 1074–1082. [\[CrossRef\]](#)
55. Charest, M.-H.; Beauchamp, G. Composting of de-inking paper sludge with poultry manure at three nitrogen levels using mechanical turning: Behavior of physico-chemical parameters. *Bioresour. Technol.* **2002**, *81*, 7–17. [\[CrossRef\]](#)
56. Rodrigues, A.; Gonçalves, A.B.; Casquilho, M.; Gomes, A.A. A GIS-based evaluation of the potential of woody short rotation coppice (SRC) in Portugal aiming at co-firing and decentralized co-generation. *Biomass Bioenergy* **2020**, *137*, 105554. [\[CrossRef\]](#)
57. Dimitriou, I.; Rosenqvist, H. Sewage sludge and wastewater fertilization of short rotation coppice (SRC) for increased bioenergy production-Biological and economic potential. *Biomass Bioenergy* **2011**, *35*, 835–842. [\[CrossRef\]](#)
58. Djomo, S.; Ac, A.; Zenone, T.; DeGroote, T.; Bergante, S.; Facciotto, G.; Sixto, H.; Ciria, P.; Weger, J.; Ceulemans, R. Energy performances of intensive and extensive short rotation cropping systems for woody biomass production in the EU. *Renew. Sustain. Energy Rev.* **2015**, *41*, 845–854. [\[CrossRef\]](#)
59. Pedersen, J.; Hafner, S.; Adamsen, A. Effectiveness of mechanical separation for reducing ammonia loss from field-applied slurry: Assessment through literature review and model calculations. *J. Environ. Manag.* **2022**, *323*, 116196. [\[CrossRef\]](#)
60. Department for Environment, Food; Rural Affairs, UK Government. Using a Mechanical Slurry Separator to Manage Your Slurry. Available online: <https://www.gov.uk/guidance/using-a-mechanical-slurry-separator-to-manage-your-slurry> (accessed on 6 November 2025).
61. Menino, R.; Castelo-Branco, M.; Duarte, E.; Fragoso, R.; Silva, I.; Cordovil, C.; Cirino, G.; Nestler, M.; Nestler, H.; Figueiredo, M.A.; et al. Roteiro para a Gestão dos Fluxos Gerados na Atividade Agropecuária. In *Technical Report*; Moreira, O., Cruz, V., Trindade, H., Eds.; Relgráfica Artes Gráficas, Lda: Benedita, Portugal, 2022; p. 212, ISBN 978-972-579-060-1. (In Portuguese)
62. Nyameasem, J.; Zutz, M.; Kluß, C.; Huf, M.; Essich, C.; Buchen-Tschiskale, C.; Ruser, R.; Flessa, H.; Olfs, H.-W.; Friedhelm, T.; et al. Impact of cattle slurry application methods on ammonia losses and grassland nitrogen use efficiency. *Environ. Pollut.* **2022**, *315*, 120302. [\[CrossRef\]](#)
63. Djomo, S.; El Kasmoui, O.; Ceulemans, R. Energy and greenhouse gas balance of bioenergy from poplar and willow: A review. *Glob. Change Biol. Bioenergy* **2011**, *3*, 181–197. [\[CrossRef\]](#)

64. Intergovernmental Panel on Climate Change. Climate Change 2021: The Physical Science Basis. In *Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Masson-Delmotte, V.P., Zhai, A., Pirani, S.L., Connors, C., Péan, S., Berger, N., Caud, Y., Chen, L., Goldfarb, M.I., Gomis, M., et al., Eds.; Cambridge University Press: Cambridge, UK, 2021; p. 2391. [[CrossRef](#)]
65. Vanbeveren, S.P.P.; Ceulemans, R. Biodiversity in short-rotation coppice. *Renew. Sustain. Energy Rev.* **2019**, *111*, 34–43. [[CrossRef](#)]
66. Custer, G.F.; Bresciani, L.; Dini-Andreote, F. Toward an integrative framework for microbial community coalescence. *Trends Microbiol.* **2024**, *32*, 241–251. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.