

Article

The Use of Organic Additives for Replanted Soil in Apple Tree Production in a Fruit Tree Nursery

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Abstract: How soil is used affects its production characteristics in the future. Under ARD (Apple Replant Disease) conditions, replanted soil's physical, chemical and biological properties deteriorate. Their improvement is possible through, for example, increasing the content of organic matter in the soil. The study aimed to assess the effect of two organic additives for replanted soil on its physical, chemical and biological properties, as well as on the vegetative growth of apple trees of the 'Gala Schniga SchniCo(s)' cultivar grafted on M.9 rootstock. The experiment was performed in 2021, in western Poland, on a nursery farm. The trees were planted in pots filled with soil from two stations: soil previously used for the production of apple trees (replanted soil) and nursery material (agricultural soil) unused for production so far. To fertilise it, three different portions of biocarbon and Carbomat Eco soil conditioner were added to the replanted soil. The experiment showed that apple trees grown on replanted soil had fewer side shoots, a smaller leaf area and a lower mass of leaves than those grown on agricultural soil. Furthermore, supplementation of replanted soil with organic additives caused a significant increase in its enzymatic activity and respiration, increased the rate of photosynthesis and improved several parameters determining the strength of vegetative growth in apple trees.



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Keywords: replanted soil; apple trees; biocoal; enzymatic activity and respiration of soil; biometric parameters of leaf; vegetative growth of trees

1. Introduction

Poland is a major apple producer in Europe and worldwide. According to FAOSTAT, in 2021, approximately 4.07 million tons of fruit of the species were produced in Poland [1]. Achieving high yield is possible thanks to the progressive intensification of orchard production, which assumes increasing the number of low-growing trees per unit area, inter alia. Trees grown on dwarf rootstocks are characterised by a relatively short lifespan [2]. All of these features increase the demand for nursery material, which are characterized by significant diversity. This is due to changing consumer expectations and the introduction of new varieties and rootstocks to the market. To meet such demands, nurseries often change plantings. Nursery production requires a multi-year break between planting changes. With the increasing deficit of areas suitable for fruit tree nurseries, the risk of establishing fruit tree nurseries by replacing old ones has grown. Under such conditions, the risk of a phenomenon known as Apple Replant Disease (ARD) has significantly increased. The term “replant disease” [3] or “soil fatigue” [4] are also found in scientific publications, mainly concerning apple orchards. Considering orchard tree species, apple trees are particularly sensitive to the effects of replant disease.

Researchers have no clear position regarding the leading cause of replant disease. However, its effects are well known. Among other things, it has been determined that the physical and chemical properties of replanted soil deteriorate and there is an increase in acidity [5], a decrease in macro and microelements [6,7] and a deterioration in biological properties, measured by enzymatic activity and respiration [8]. Consequently, the vegetative growth of plants is weakened [9,10], which reduces the profitability of production [11].

There are several ways to mitigate the effects of replant disease. Examples include soil fumigation, using intercrops or the introduction of crop rotation. Abiotic factors can contribute to ARD. Therefore, one of the ways to reduce of ARD is by enriching the soil with nutrients. Both mineral and organic fertilisers are used for that purpose. Despite their undoubted advantages (high efficiency, speed of action), mineral fertilisers are costly and energy-intensive. Mineral fertilisation, which is not fully used by the plant, leads to decreased microbiological diversity in the soil [12]. Their excessive use can lead to an increase in soil acidity, a violation of the balance of nutrients or a deterioration in the quality of the obtained yield [5,13].

Apart from basic mineral fertilisation, various biostimulants, including organic soil additives, have become increasingly popular in horticultural production. These include amino acids of animal and plant origin, animal fertilisers in solid or liquid form, compost and humic acids [14,15]. The latter are the main components of soil humus. According to the literature, humic acids can increase the rate of photosynthesis [16,17], improve soil structure and water-air relations in soil [18], increase plant resistance to abiotic stress and even increase the activity of hormones and antioxidants [19]. Humic acids not only increase the content of organic matter in soil [20], but also, by stimulating the proliferation of beneficial soil microorganisms, facilitate the mineralisation of soil. Furthermore, the developed nutrients are easily absorbed by plants [21–24].

As mentioned earlier, supplementing replanted soil with organic matter using organic additives may enable faster restoration of its productive properties. Therefore, the study aimed to assess the effect of two organic additives for replanted soil on the change of its physical, chemical and biological properties, as well as on the growth parameters of apple trees.

2. Materials and Methods

2.1. Study Sites and Experimental Design

The experiment was performed in 2021, on a nursery farm in western Poland (52°25′45.553 N, 17°11′32.755 E). The study material was apple trees of the ‘Gala Schniga SchniCo(s)’ cultivar grafted on M.9 rootstock obtained from hand grafting in winter, planted in 8-litre containers. The containers were filled with soil taken from two different stations: from a field where agricultural crops had been grown for the last 10 years (the so-called agricultural soil) and from an old nursery where apple trees had been grown for 3 years (the so-called replanted soil). The replanted soil used in the experiment was not prepared for new nursery plantings in any way.

The analysis of the physical and chemical properties of both types of soil is shown in Table 1.

The following eight treatments were used in the experiment: treatment 1—agricultural soil (control); treatment 2—replanted soil; treatments 3, 4 and 5—replanted soil with the addition of the so-called biocarbon in the amounts of 5, 10 and 15% of the pot volume; treatments 6, 7 and 8—replanted soil with the addition of Carbomat Eco brown lignite in the amounts of 15, 30 and 45% of the pot volume. Each treatment was represented by 10 repetitions (pots).

The biocarbon used in the experiment was obtained by anaerobic decomposition of wood pellets at 400–500 °C. Based on the analysis of the chemical composition of biocarbon, it contains humic acids and macronutrients (in % dm), such as: N 0.17; K 0.11; Ca 0.23; Mg 0.06, as well as micronutrients (in mg kg⁻¹ dm): Zn 30.9; Cu 2.57; Mn 103; Fe 194; B 8.42. Salinity is < 0.24 g Na Cl l and pH is 7.68. The second soil additive, Carbomat Eco

(pH 6.0–6.5), is an organic agent made from soft brown coal lignite. Apart from humic acids, it also contains macro- and micronutrients. The agent is designed to improve soil's physical, chemical and biological properties, especially soil with low humus content. Carbomat Eco's task is to stimulate the activity of beneficial soil microflora and maintain the correct soil pH.

Table 1. Physicochemical and biological properties of the soil used in the experiment.

Properties of the Soil	Agricultural Soil	Replanted Soil
pH (H ₂ O)	5.7	5.4
Volumetric weight (g dm ^{−3})	1.69	1.71
Salinity (g Na Cl dm ^{−3})	0.16	0.13
Mineral content (mg dm ^{−3}): N-NO ₃	16	11
P	30	51
K	143	107
Ca	230	184
Mg	54	51
Cl	2	1
Dehydrogenase activity (cm ³ H ₂ 24 h ^{−1} kg ^{−1} dm)	0.26	0.21
Protease activity (mg tyrosine h ^{−1} kg ^{−1} dm)	3.13	1.83
Respiration (CO ₂ mg kg ^{−1} 24 h ^{−1})	24.86	28.79

Necessary care during the growing season was carried out in accordance with the recommendations for the commercial production of nursery material. Meteorological conditions during the research were analysed based on the measurements of a meteorological station located several kilometers from the place of the experiment. The average annual precipitation in 2021 (411.6 mm) was significantly lower than in the year preceding the research (438.4 mm). A very high amount of precipitation occurred in June (94.6 mm), but throughout the growing season, there were periodic water shortages. In addition, the average annual temperature in 2021 (9.9 °C) was lower than that in 2020 (10.3 °C), but higher than the average for the region (8.6 °C).

2.2. Measurements and Observations

2.2.1. Measurements of the Soil

During the experiment, the following analyses of the physical and chemical properties of the soil were taken: the content of macroelements (N-NO₃, P, K, Ca, Mg and Cl) and microelements (Zn, Cu, Mn, Fe), acidity, salinity and bulk density; total content of phenolic compounds. At the end of the growing season, soil samples were taken from each repetition (container) to analyse the physical, chemical and biological properties. After mixing them, one sample representative of the treatment was obtained, weighing approximately 0.5 kg. The collected samples were analysed for mineral content using the universal method. Extraction of macroelements was carried out in 0.03 M CH₃COOH with a quantitative 1:10 proportion of substrate to extraction solution. The following macroelements were determined: N-NO₃—by micro-distillation; P—calorimetrically with ammonium vanadomolibdate; K, Ca—photometrically; Mg—by atomic absorption spectrometry (AAS); Cl—nephelometrically with AgNO₃. Micronutrients were extracted from the soil with Lindsay's Solution and were determined using the AAS method. Soil acidity was determined using the potentiometric method.

Biological properties of soil included: the activity of enzymes (proteases and dehydrogenases) and soil respiration. Protease activity (in mg tyrosine h^{−1} kg^{−1} dm of soil) was determined using the spectrophotometric method according to Ladd and Butler [25], using 1% sodium caseinate solution as substrate. The abovementioned properties were

determined after one-hour incubation of the samples at 50 °C, using a spectrophotometer, at a wavelength of 578 nm. The activity of dehydrogenases (in $\text{cm}^3 \text{H}_2 \text{ 24 h}^{-1} \text{ kg}^{-1} \text{ dm}$ of soil) was determined using the colorimetric method according to Öhlinger [26], using a 1% solution of TTC (triphenyl tetrazolium chloride). The above was determined after 24-h incubation of the samples at 30 °C, using a spectrophotometer, at a wavelength of 485 nm (TTC test). Soil respiration ($\text{CO}_2 \text{ mg kg}^{-1} \text{ 48 h}^{-1}$) was measured based on the amount of released CO_2 , using the absorption method according to Gołębiowska and Pędziwilk [27]. Finally, the soil's total content of phenolic compounds in the soil was spectrophotometrically determined using Folin's reagent. All of the above properties were determined four times for each treatment.

2.2.2. Biometric Measurements and Analyses of Leaves

Biometric measurements of the leaves included: the leaf area (cm^2), its length (cm), width (cm) and weight (g). In addition, the photosynthetic activity of the leaves as well as the content of macroelements (N_{org} ; P_2O_5 ; K_2O ; CaO ; MgO (in % dm) and microelements (Zn, Cu, Mn, Fe, B (in $\text{mg kg}^{-1} \text{ dm}$) in the leaves were measured. To perform biometric measurements, the leaves were collected at the end of September, after the intensive growth of the trees was over. The leaf mass was measured for four repetitions (10 leaves per repetition). They were weighed with an accuracy of 0.1 g. The weighed leaves were scanned, and then, using Digshape software (ver.1.9.19, Cortex Nowa, Bydgoszcz, Poland), their area was calculated. To analyse the macro- and micronutrient content in the leaves of the trees, 200 leaves were collected from each treatment and dried using a laboratory blow-dryer at 65 °C. After drying, the samples were ground using a laboratory grinder. The ground leaves were mineralised in a Turbotherm mineraliser using sulphuric acid (H_2SO_4). N, P, K, Ca and Mg content was determined. The Kjeldahl method was used to determine the content of N, the vanadium and molybdenum method was used to determine the P content and the atomic absorption method for K, Ca and Mg using a Zeiss-Jena AAS-5 apparatus (Carl Zeiss Jena GmbH, Jena, Germany).

The gas exchange intensity of the apple tree leaves was analysed based on the following parameters: net photosynthesis intensity ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), leaf transpiration coefficient ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and intercellular carbon dioxide concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$). A portable gas exchange analyser LCiT ADC (BioScientific Ltd., Rickmansworth, UK) was used for the measurements, using a wide chamber with an area of 6.25 cm^2 . The measurements were taken under similar conditions: a CO_2 flow rate of $400 \mu\text{mol min}^{-1}$, a photosynthetic photon flux density (PPFD) of $1200 \text{ mol (photon) m}^{-2} \text{ s}^{-1}$ and a chamber temperature of 23 to 26 °C. The measurements were taken twice: in the middle of June and in the middle of August, six times for each treatment. They were taken around noon, using randomly selected, healthy, well-developed leaves.

2.2.3. Measurements of the Growth Parameters of Apple Trees

The power of the growth of the trees was assessed by measuring: the annual growth of the diameter of their trunks, the height of the trees, the number and length of side shoots, the mass of the main shoot and side shoots, as well as the mass and length of the roots. The diameter of the tree trunks (mm) was measured 10 cm above the grafting area, in the spring, immediately after planting, and in the autumn after the end of vegetation. The height of the trees was measured from the root neck to the top of the main shoot. The number of side shoots (over 2 cm long) was determined and their total length was calculated. The aboveground part of the trees (g) was measured, and the mass of the roots (g) and length were determined. The root system of the trees, after rinsing the roots with water under constant pressure to remove the soil, was weighed with an accuracy of 1 g. The power of the growth of the aboveground and underground parts was determined for all trees in the treatment (10 repetitions).

2.3. Statistical Analysis

The experiment results were subjected to univariate statistical analysis, in which the factors were the used combinations, using the STATISTICA 12.1 software. Statistical analysis was performed using the analysis of variance method, and the differences between the means were assessed using Duncan's test at the significance level of $\alpha = 0.05$.

3. Results and Discussion

3.1. Physical, Chemical and Biological Properties of Soil

Under ARD conditions, soil productivity deteriorates. The previous way of using the soil analysed in the experiment changed some of its physical and chemical properties. The volumetric weight of the soil, its salinity and the content of phenolic compounds did not significantly differ (Table 2). The level of acidity and the amount of organic matter varied. The pH value of the replanted soil was significantly lower than that of the agricultural soil (Table 2). The above confirms other researchers' conclusions on the replanted soil's high acidity [5]. The organic matter content in the agricultural soil (1.87%) was higher than that in the replanted soil (1.63%).

Table 2. Physico-chemical properties of the soil.

Treatments	Soil Bulk Density (g dm ⁻³)	pH (H ₂ O)	Salinity (g NaCl dm ⁻³)	Organic Matter (%)	Phenolic Compounds (mg g ⁻¹ d.m.)
Agricultural soil	1 710 d ¹	7.8 b	0.31 b	1.87	13.19 a
Replanted soil	1 760 d	6.7 a	0.47 b	1.63	14.72 a
Biocoal 5%	1 670 c	7.8 b	0.20 a	2.78	16.13 b
Biocoal 10%	1 620 c	7.4 ab	0.38 b	3.54	23.16 c
Biocoal 20%	1 580 b	6.3 a	0.24 a	3.86	27.83 c
Carbomat Eco 15%	1 580 b	8.2 b	0.19 a	5.56	14.78 a
Carbomat Eco 30%	1 470 ab	6.9 a	0.15 a	7.58	16.89 b
Carbomat Eco 45%	1 350 a	6.1 a	0.19 a	8.67	17.04 b

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

Supplementing the replanted soil with the two types of organic additives, regardless of their amount, significantly reduced the volumetric weight of the soil and the level of its salinity. Carbomat Eco was more effective than biocarbon in that respect. In the treatments with the addition of Carbomat Eco, in particular in the amount of 30% of the substrate volume, compared to the replanted soil with no additives, the salinity of the soil decreased more than three times (from 0.47 to 0.15 g NaCl dm⁻³) (Table 2). High soil salinity, causing its alkalisation, reduces the assimilation by plants of macro- and microelements and limits its enzymatic activity. The experiment showed significant differences in the content of phenolic compounds in the soil depending on the treatment. Their content was higher in the replanted soil with added carbon than in the replanted soil with no additives. Biocarbon was a particular case. In the treatments with biocarbon, an increase in phenolic compounds content by approximately 90% (from 14.79 to 27.83 mg g⁻¹ dm) was observed (Table 2). In the treatments with Carbomat Eco, the increase in the content of the abovementioned compounds was a dozen or so per cent (volume in the substrate 30 % and 45%) or insignificant (volume 15%).

The content of the five macronutrients under study in replanted and agricultural soil did not significantly differ. This is in contrast to the experimental results obtained by the authors of earlier studies [8], when the macronutrients content in replanted soil was considerably lower than that in agricultural soil. In the treatments, where replanted soil was supplemented with organic additives, N-NO₃ content decreased. In the soil with the addition of biocarbon at 20% of the substrate volume, the content of that element (48 mg dm⁻¹) was even 3.5 times lower than in the control treatment (agricultural soil)

(180 mg kg⁻¹) (Table 3). The decrease in N-NO₃ content of the soil was equally significant after adding Carbomat Eco, especially at 45% of the substrate volume. Furthermore, compared to replanted and agricultural soil, a lower content of P and K was also noticed in the treatments with its use.

Table 3. The content of macroelements in the soil (mg dm⁻³).

Treatments	N-NO ₃	P	K	Ca	Mg	Cl
Agricultural soil	180 b ¹	96 c	462 c	512 ab	144 a	32 b
Replanted soil	160 b	56 b	240 b	348 a	121 a	36 b
Biocoal 1 5%	82 a	70 c	303 b	499 ab	182 ab	14 a
Biocoal 10%	75 a	89 c	410 c	461 a	213 b	12 a
Biocoal 20%	48 a	131 d	507 d	656 bc	259 b	12 a
Carbomat Eco 15%	78 a	30 ab	149 a	481 a	114 a	20 ab
Carbomat Eco 30%	102 ab	19 a	123 a	745 c	150 ab	31 b
Carbomat Eco 45%	68 a	13 a	67 a	634 b	113 a	10 a

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

The decrease in N, P and K content in the soil with the addition of Carbomat Eco may have resulted from a more intensive uptake of those elements by plants. However, in the replanted soil with the addition of biocarbon, especially at 10 and 20%, P, K and Mg content increased compared to their content in the replanted soil with no additives (Table 3).

In the experiment, the number of micronutrients in the soil was less diverse than that of macronutrients. The Cu, Mn and Fe content in the replanted soil did not significantly differ from their content in the agricultural soil. The difference was only noticed in the case of Zn, the content of which in treatments with the application of biocarbon significantly decreased (several times compared to the control). The reduction was greater with an increase in its content in the soil (Table 4). Even though both organic additives used in the experiment contained macro- and microelements, their effect on the content of nutrients in the soil was relatively small. After adding biocarbon to the replanted soil, regardless of its amount, a significant decrease in the content of Mn was observed. The number of other micronutrients in the soil did not significantly change. A slightly different effect was caused by adding Carbomat Eco to the replanted soil. In the treatment with the addition of Carbomat Eco lignite, irrespective of its amount, there was a significant increase in the content of Zn and Fe in the soil. The content of Mn and Cu did not significantly change (Table 4).

Table 4. The content of microelements in the soil (in mg dm⁻³).

Treatments	Zn	Cu	Mn	Fe
Agricultural soil	5.02 b ¹	2.09 c	55.34 b	113.08 a
Replanted soil	4.32 a	1.95 bc	72.75 b	113.88 a
Biocoal 5%	3.88 a	1.79 b	10.81 a	95.55 a
Biocoal 10%	4.14 a	1.79 b	11.86 a	98.50 a
Biocoal 20%	4.10 a	1.58 b	9.92 a	142.90 a
Carbomat Eco 15%	5.31 b	1.46 ab	50.40 b	357.61 b
Carbomat Eco 30%	5.87 b	1.43 ab	76.33 b	358.10 b
Carbomat Eco 45%	5.68 b	0.92 a	48.33 b	377.55 b

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

The level of biological activity of soil is measured by its enzymatic activity. The higher the activity of soil enzymes, the higher the rate of mineralisation of organic compounds, and thus, the amount of macro- and microelements available to the plants. Some of the most important soil enzymes are dehydrogenases and proteases. These enzymes are involved in the soil's biochemical cycle of carbon, nitrogen and phosphorus [28]. Dehydrogenases are particularly important as they are the source of information regarding the activity of the soil microflora [29]. In the experiment, the biological properties of the replanted soil significantly differed from those of the agricultural soil. For example, dehydrogenase activity was more than three times lower (0.22 and $0.78 \text{ cm}^3 \text{ H}_2 \text{ 24 h}^{-1} \text{ kg}^{-1} \text{ dm}$ of soil) (Table 5). The level of respiration of replanted and agricultural soil was less differentiated.

Table 5. Enzymatic and respiratory activity of the soil.

Treatments	Dehydrogenase (in $\text{cm}^3 \text{ H}_2 \text{ 24 h}^{-1} \text{ kg}^{-1} \text{ dm}$)	Protease (in $\text{mg Tyrosine h}^{-1} \text{ kg}^{-1} \text{ dm}$)	Respiratory Activity (CO_2 in $\text{mg kg}^{-1} \text{ 24 h}^{-1}$)
Agricultural soil	0.78 bc^1	2.36 a	37.78 cd
Replanted soil	0.22 a	2.08 a	26.56 a
Biocoal 5%	0.59 b	3.52 b	30.34 ab
Biocoal 10%	0.21 a	3.61 b	34.73 bc
Biocoal 20%	0.24 a	3.65 bc	33.16 bc
Carbomat Eco 15%	0.36 a	3.96 c	40.84 d
Carbomat Eco 30%	0.65 b	4.76 cd	47.69 e
Carbomat Eco 45%	0.93 c	5.51 c	52.97 f

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

The lower activity of dehydrogenases in the replanted soil may have resulted from the insufficiently effective work of soil microorganisms, which decreases once the soil acidity is reduced [30]. The replanted soil used in the experiment was more acidic than the agricultural soil (Table 2). However, the authors did not show any significant differences in the activity of soil proteases depending on the previous way of using the soil.

Organic matter in soil is the source of nutrients and energy necessary for the functioning of soil microorganisms [31]. When it decomposes, the microorganisms produce enzymes, thus determining the level of soil productivity [32]. The indication of the level of activity of soil microorganisms is soil respiration [31,33]. Adding the two organic additives to the replanted soil significantly improved that parameter. In the treatments with the addition of biocarbon in the amounts of 10 and 20%, compared to the replanted soil with no additives, the respiration of the soil increased by 30 and 25%, respectively (Table 5). A better result was obtained in the treatments by adding Carbomat Eco. With the increase in its amount in the substrate, the replanted soil respiration significantly increased—from approximately 50% (volume 15%) to almost 100% (volume 45%). The respiration level was much higher than that of the agricultural (control) soil.

Similar results were obtained when soil enzymatic activity was analysed. As a result of adding both biocarbon and Carbomat Eco lignite to the replanted soil, protease activity in the soil significantly increased. The addition of Carbomat Eco was more effective in this regard. In the replanted soil with Carbomat Eco added at 45% of the substrate volume, an over 2.5-fold increase in protease activity was obtained compared to the replanted soil with no additives (5.51 and $2.08 \text{ mg of tyrosine h}^{-1} \text{ kg}^{-1} \text{ dm}$, respectively) (Table 5). In the treatment with biocarbon added in the amount of 20% of the substrate volume, an increase in the activity of proteases was found at approximately 75%. Once the amount of the additive was increased from 5 to 20% (biocarbon) and from 15 to 45% (Carbomat Eco), no significant increase in the activity of proteases was observed in the replanted soil.

The level of activity of soil dehydrogenases varied depending on the treatment. When biocarbon was added to the replanted soil in the amount of 5% of the volume of the substrate, a significant (more than twofold) increase in the activity of the abovementioned

enzyme was found. As in the case of protease activity and soil respiration, the best results were obtained in the treatments with the addition of Carbomat Eco lignite. At 30% of its content in the replanted soil, the activity of dehydrogenases, compared to the replanted soil with no additives, increased almost three times, and at 45%, it increased more than four times (0.65 and 0.22 and 0.93 $0.22 \text{ cm}^3 \text{ H}_2 \text{ 24 h}^{-1} \text{ kg}^{-1} \text{ dm}$, respectively) (Table 5). The obtained results confirm the conclusions of the previous studies and indicate the positive effect of humic acids on both the enzymatic activity and respiration of soil [8,34].

3.2. Apple Tree Leaf Parameters

The leaf minerals can be used as an indicator of plant nutrition levels. However, several factors can influence the diversity of mineral content in plant leaves. These include, inter alia, their content in the soil, weather conditions, the yield level and the age of trees, as well as the cultivation conditions before planting the trees. In the experiment, depending on the treatments, the content of macroelements in the leaves of the apple trees (in % dm) was: N—from 2.3 to 2.52; P—from 0.15 to 0.40; K—from 1.44 to 3.9; CaO—from 0.69 to 1.14; Mg—from 0.17 to 0.30. The content of macroelements in the leaves of the apple trees grown on the agricultural and replanted soil did not significantly differ (Table 6).

Table 6. The content of macronutrients (% dm) in apple tree leaves.

Treatments	Norg.	P	K	Ca	Mg
Agricultural soil	2.40 a ¹	0.19 b	1.99 c	1.03 bc	0.30 c
Replanted soil	2.30 a	0.19 b	2.08 c	1.03 bc	0.27 bc
Biocoal 5%	2.40 ab	0.22 c	2.99 d	0.92 b	0.24 b
Biocoal 10%	2.40 ab	0.19 b	3.04 d	0.92 b	0.26 bc
Biocoal 20%	2.40 ab	0.26 d	3.90 e	0.69 a	0.17 a
Carbomat Eco 15%	2.40 ab	0.15 a	1.44 a	1.14 c	0.27 bc
Carbomat Eco 30%	2.52 b	0.17 a	1.81 b	0.97 bc	0.25 b
Carbomat Eco 45%	2.52 b	0.40 a	1.83 b	0.92 b	0.23 b

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

Supplementing the replanted soil with organic additives had a varied effect on the content of the macroelements under study. In the leaves of the apple trees grown on the replanted soil with the addition of biocarbon in the amounts of 10 and 20% of the substrate volume, compared to the replanted soil with no additives, the content of N, Ca and Mg did not change (Table 6). However, the content of P and K increased. In the treatments with the addition of Carbomat Eco, the content of Ca and Mg in the leaves did not change, the content of K and P significantly decreased (especially at 30 and 45% volume) and the content of N significantly increased. The increase in the macronutrient contents in the leaves of apple trees under the influence of humic acids can be explained by the fact that they increase the permeability of cell membranes, which accelerates the absorption of nutrients by the roots and their accumulation in plant tissues.

In the experiment, depending on the treatment, the content of micronutrients in the leaves of the apple trees (in mg kg dm⁻¹) was: Zn—from 11.3 to 14.2; Cu—from 3.96 to 5.31; Mn—from 57.7 to 337; Fe—from 139 to 178; B—from 17.35 to 29.05 (Table 7).

The content was higher (Fe, Mn), comparable (Cu, B) or lower (Zn) than in the experiment performed by Sosna et al. [35], presenting the micronutrient contents in the leaves of apple trees grafted on M.9 rootstock. Generally, supplementing the substrate with organic additives had little effect on the content of microelements in the leaves of apple trees. Regardless of the type of additive, there were no significant differences in the Zn and Fe content of the leaves. This result differs from the results obtained in the experiment conducted by Abourayya et al. [36], which showed an increase in the Fe and Zn

content in almond leaves treated with humic acid. In the leaves of the apple trees grown on the replanted soil with the addition of Carbomat Eco, compared to those grown on the replanted soil with no additives and agricultural soil, the content of B significantly increased. With the addition of biocarbon, the content of Cu also increased (Table 7).

Table 7. The content of micronutrients (mg kg dm^{−1}) in apple tree leaves.

Treatments	Zn	Cu	Mn	Fe	B
Agricultural soil	12.4 a ¹	5.10 b	202 b	155 ab	19.80 a
Replanted soil	12.6 a	4.68 a	337 b	155 ab	17.35 a
Biocoal 5%	13.1 a	5.31 b	96 a	148 a	17.60 a
Biocoal 10%	11.8 a	5.10 b	68 a	154 ab	19.70 a
Biocoal I 20%	11.3 a	5.08 b	58 a	139 a	19.75 a
Carbomat Eco 15%	14.2 a	4.48 a	284 b	178 b	24.75 b
Carbomat Eco 30%	12.7 a	4.74 a	232 b	146 a	28.20 b
Carbomat Eco 45%	12.0 a	3.96 a	202 b	138 a	29.05 b

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

The experiment showed the significant influence of the previous method of using the soil on the parameters of the apple trees' leaves, particularly in the case of the replanted soil, compared to the agricultural soil. For example, the leaf area of the apple trees grown on the replanted soil (28.41 cm²) was approximately 70% smaller than those in the control variant (49.27 cm²) (Table 8). Furthermore, a significant difference (from 50% to 70%, depending on the number of organic additives) was also observed in other parameters, such as the length of the leaves and their mass. Thus, the earlier conclusion of the study's authors was confirmed—the biometric parameters of the leaves of apple trees grown on replanted soil were adversely affected [37].

Table 8. Effect of substrate on biometric parameters of the leaves of apple trees.

Treatments	Leaf Area (cm ²)	Leaf Length (cm)	Leaf Width (cm)	Leaf Weight (g)
Agricultural soil	49.27 b ¹	10.89 b	4.94 ab	6.77 b
Replanted soil	32.41 a	7.25 a	4.56 a	4.43 a
Biocoal 5%	45.67 b	10.25 b	5.09 ab	4.88 a
Biocoal 10%	44.69 b	9.77 b	4.58 a	4.22 a
Biocoal I 20%	47.00 b	11.06 b	5.00 ab	3.94 a
Carbomat Eco 15%	55.01 bc	10.53 b	5.57 ab	7.23 b
Carbomat Eco 30%	72.21 d	12.34 b	5.96 b	7.81 bc
Carbomat Eco 45%	65.31 cd	11.51 b	5.81 b	8.49 c

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

Eisa et al. [38] and Suppels et al. [16] reported that treating apple trees with biostimulants containing humic acids increased the leaf area. The authors of the experiment confirm the above conclusion. In addition, supplementing the replanted soil with organic additives significantly improved the biometric parameters of the leaves of the apple trees. In that respect, Carbomat Eco was more effective than biocarbon. Even with its smallest amount in the substrate of 15%, the leaf area of the apple trees (55.01 cm²) was almost twice as large as that in the replanted soil with no additive combination (28.41 cm²) (Table 8). In the variant with 30% of the substrate volume, the difference was over 2.5 times. A further increase in the amount of Carbomat Eco to 45% did not significantly impact the leaf area of

the trees. Similar results were obtained when the leaf mass of the apple trees was analysed. Again, adding Carbomat Eco lignite to the replanted soil at 15% of the substrate volume increased the average leaf mass of the apple trees grown on the soil with no additives from 4.43 g to 7.23 g. A further significant increase in average leaf mass to 8.49 g was possible when the amount of the abovementioned additive in the substrate was increased to 45%. Once biocarbon was added to the replanted soil, the leaf area of the trees also increased. Compared to the replanted soil with no additives, the difference was less than 70% (28.41 and 47.0 cm² in the variant with 20% substrate volume) (Table 8). Notably, the amount of biocarbon in the substrate had no significant effect on the leaf area.

Another parameter that positively responded to the addition of biocarbon to the soil was the length of the leaves. Compared to the replanted soil with no additive treatment, the difference in length ranged from approximately 30% (10% of the substrate volume) to about 20% (20% volume). The analysis of leaf width and leaf mass showed no significant difference between the treatments with the addition of biocarbon, regardless of its amount in the substrate, and the replanted soil with no additives (Table 8).

Well-developed leaves enable the proper course of photosynthesis, which translates into better plant growth. In the experiment, the intensity of gas exchange in the leaves of the apple trees was as follows: the amount of intercellular CO₂ (vpm) from 217.10 to 252.70; transpiration coefficient (mmol m⁻² s⁻¹) from 2.05 to 3.09; rate of photosynthesis (μmol m⁻² s⁻¹) from 11.70 to 17.00. The intensity of photosynthesis in the leaves of the apple trees was higher than in the leaves of pear trees—from 5.6 to 8.6 μmol m⁻² s⁻¹ [39]. The previous method of using soil significantly affected the gas exchange intensity of the apple trees' leaves. In the leaves of the trees grown on the replanted soil, the transpiration rate E (2.05 mmol m⁻² s⁻¹) was approximately 30% lower than in the case of the trees grown on agricultural soil (2.72 mmol m⁻² s⁻¹) (Figure 1). Significant differences were also noticed when the rate of photosynthesis was analysed. That rate, in the agricultural soil variant, was approximately 45% higher than in the replanted soil (17.0 and 11.7 μmol m⁻² s⁻¹, respectively).

Supplementing the replanted soil with organic additives significantly affected the intensity of the gas exchange of apple trees leaves. Compared to the trees grown on the replanted soil with no additives, there was a several per cent decrease in the amount of intercellular CO₂ in the leaves of the trees grown on the soil supplemented with Carbomat Eco. Such a difference was not observed in the combination using biocarbon in the amounts of 5 and 10% of the substrate volume. The transpiration coefficient in the leaves of the trees grown on the replanted soil with the addition of Carbomat Eco significantly increased, regardless of its amount in the substrate. When biocarbon was used in 10% and 20% of the volume, the differences between those treatments and the replanted soil with no additives were more significant, reaching 50% (3.09 and 2.05 mmol m⁻² s⁻¹, respectively). The rate of photosynthesis in the leaves of the apple trees in the treatments with the addition of biocarbon to the soil in the amounts of 10% and 20% of the substrate volume was several per cent higher than the trees grown on the replanted soil with no additives (Figure 1). A significant increase in the intensity of photosynthesis in the leaves was also observed for the treatment in which Carbomat Eco lignite was used. Furthermore, with its 15% content in the substrate, meant that the measurement results of that parameter (13.72 μmol m⁻² s⁻¹) were significantly higher than in the case of the replanted soil with no additives (11.70 μmol m⁻² s⁻¹). A further increase in the amount of Carbomat Eco in the substrate did not impact the rate of photosynthesis in the leaves of the apple trees. It should be noted that the intensity of photosynthesis in the leaves of the apple trees grown on the replanted soil, despite its supplementation with organic additives, was significantly lower than in the control variant with agricultural soil. The fact that the use of biostimulants increases the intensity of photosynthesis in the leaves of apple trees has also been confirmed by other researchers [16,17]. The change in the intensity of the photosynthesis in the leaves of the trees could have been influenced by the content of some macroelements in the replanted soil supplemented with organic additives responsible for the correct course of

that process. An example of such a component is Mg; the content in the replanted soil with the addition of biocarbon was significantly higher than in the soil with no additives (Table 3).

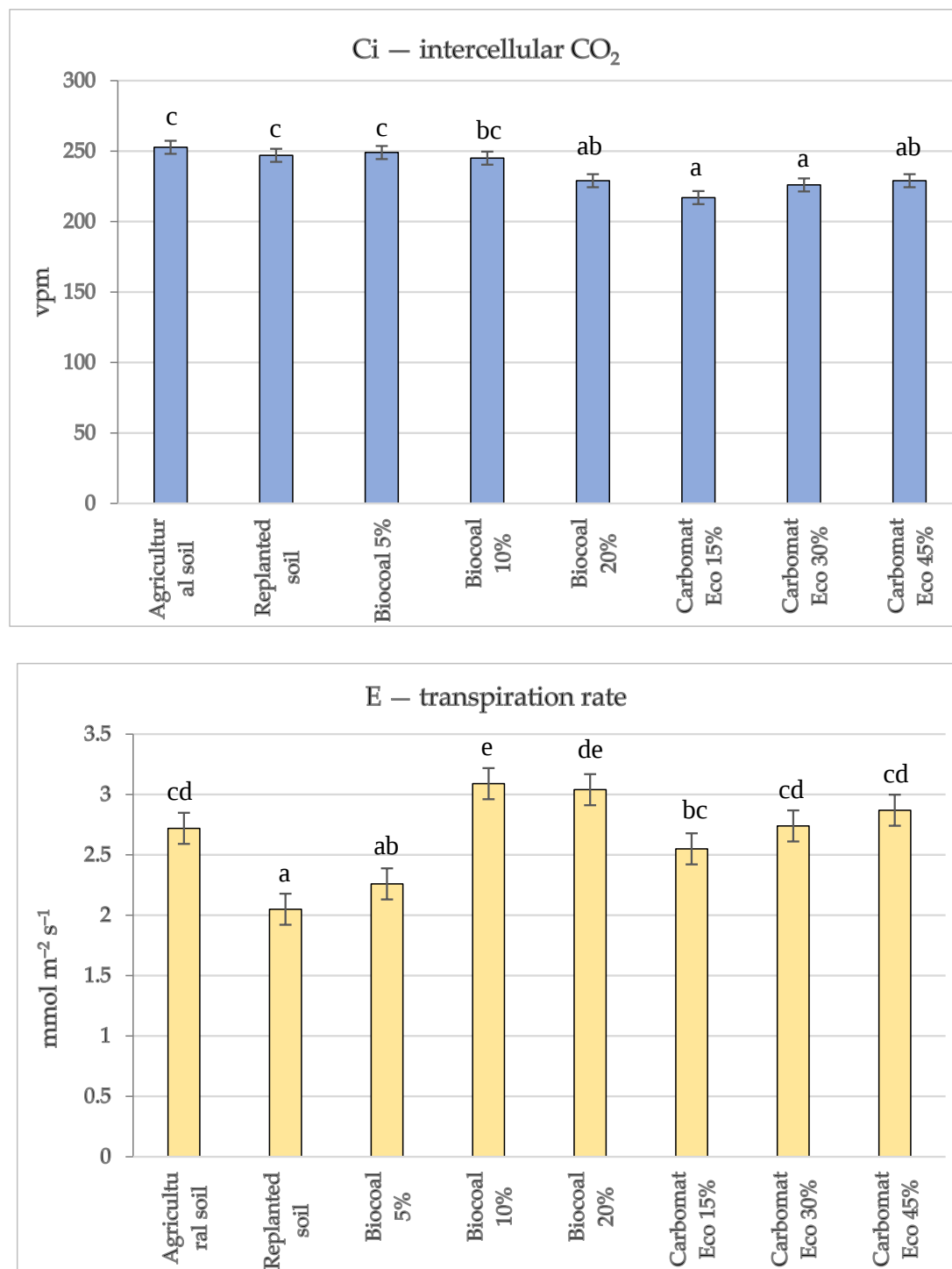


Figure 1. Cont.

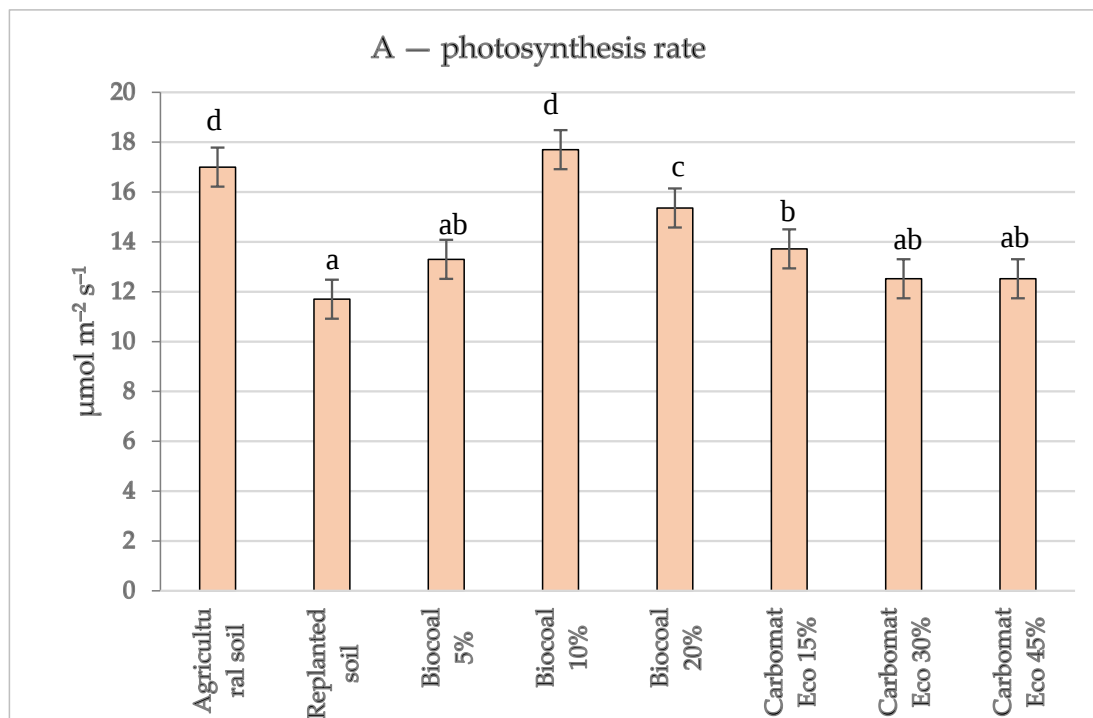


Figure 1. Gas exchange intensity in apple tree leaves. The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

Another factor that influenced the intensity of gas exchange in the leaves of apple trees was the timing of the measurements. The amount of intercellular CO_2 (C_i), the transpiration rate (E) and the rate of photosynthesis (A) were significantly lower in the first period (middle of June) than in the second period (middle of August) (Table 9).

Table 9. The intensity of gas exchange in the leaves of apple trees depending on the timing of the measurements.

Parameters	Middle of June	Middle of August
C_i —intercellular CO_2 (vpm)	225.47 a ¹	248.75 b
E —transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$)	2.32 a	3.07 b
A —photosynthesis rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	12.92 a	15.64 b

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

3.3. The Growing Power of Apple Trees

The previous method of using the soil had a varied effect on the power of vegetative growth of the apple trees. For example, trees of similar height (89.65 and 92.35 cm) grew on replanted and agricultural soil (Figure 2). However, based on earlier studies [37], the differences in apple tree growth depending on how the soil was used were much more pronounced.

In the experiment, the authors noted significant differences in the increase in the diameter of the tree trunks and the total increase in the growth of side shoots. The increase in the diameter of the trunks of the trees grown on the agricultural soil was 0.24 mm greater than that those grown on the replanted soil. On the other hand, the total growth of the side shoots of the trees grown on the replanted soil (56.98 cm) was several dozen cm smaller than of those grown on the agricultural soil (71.30 cm) (Table 10). Weaker growth of plants under ARD conditions has also been reported by other researchers [10,11,40–43]. Weaker

vegetative growth of trees grown on replanted soil may result from the deterioration of the growth parameters of the root system of plants grown under such conditions. It manifests in the formation of root necrosis, a reduction in the number of fine hair-like roots or slower growth, inter alia [44,45].

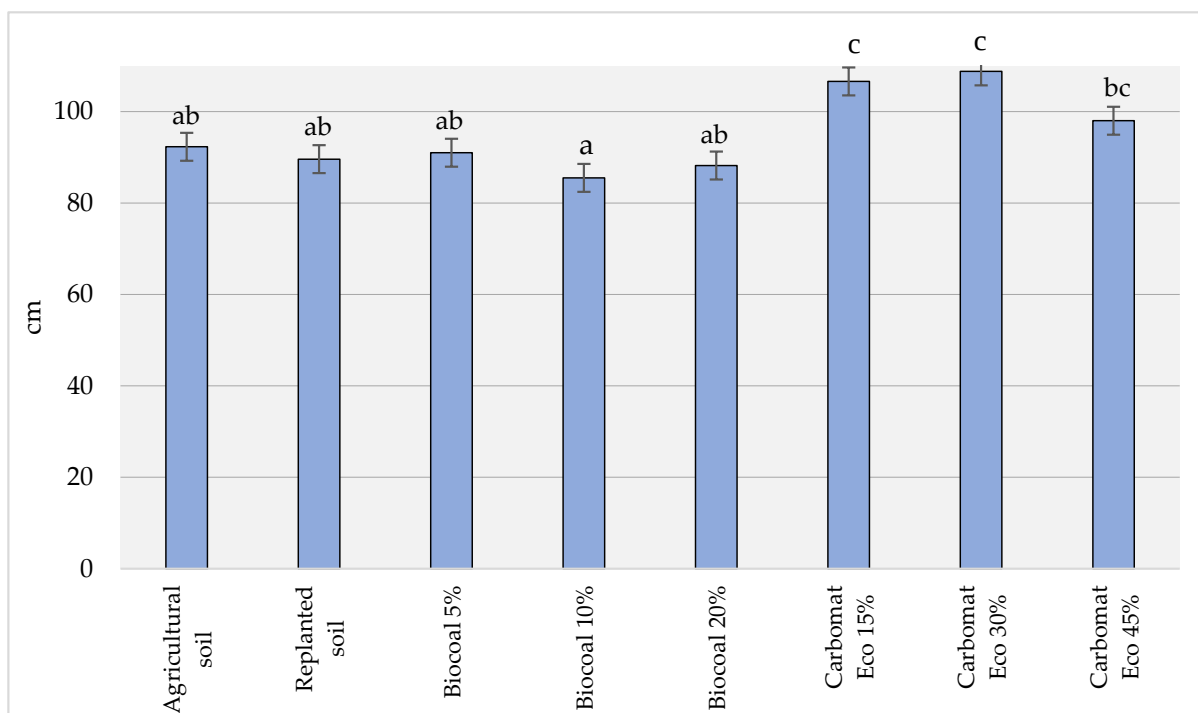


Figure 2. Effect of substrate on height of the apple trees. The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

Table 10. Effect of substrate on the growing power of apple trees.

Treatments	Increase in the Diameter of the Tree Trunks (mm)	Number of the Side Shoots	Total Mass of the Side Shoots (g)	Total Growth of the Side Shoots (cm)
Agricultural soil	1.24 bcd ¹	4.8 abc	14.03 a	71.3 bc
Replanted soil	1.00 abc	4.6 ab	9.12 a	56.98 ab
Biocoal 5%	0.60 a	4.0 a	9.02 a	33.19 a
Biocoal 10%	0.77 ab	4.8 abc	6.73 a	36.69 a
Biocoal 20%	0.80 ab	4.2 a	6.70 a	35.81 a
Carbomat Eco 15%	1.76 d	4.3 abc	25.08 b	68.24 bc
Carbomat Eco 30%	1.31 bcd	6.7 c	30.00 c	95.79 c
Carbomat Eco 45%	1.55 cd	6.5 c	41.30 d	87.64 bc

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

The addition of biocarbon to the replanted soil, regardless of its amount in the substrate, did not significantly affect the vegetative growing power of the apple trees. Their height, the increase in trunk diameter and the number and total growth of shoots of the trees grown on such a substrate did not significantly differ from the results of the measurements taken for the replanted soil with no additives treatments. However, a significant improvement in the vegetative growth of apple trees was observed when Carbomat Eco lignite was used. In such cases, each of the analysed parameters determining the growing power of the apple trees was significantly higher than in the replanted soil with no additives. For example, the trees were approximately 20% higher (30% of the volume of the substrate) (108.8 and 89.65 cm) (Figure 2), and the total growth of their side shoots was approximately 60% (95.79

and 56.98 cm) (Table 10). The trees grown in the soil supplemented with Carbomat Eco were even higher than those grown in the control combined with the agricultural soil. The best result was obtained when the mass of the side shoots of the apple trees was analysed. In the treatment with the addition of Carbomat Eco to the replanted soil in the amount of 30% of the substrate volume, the measurement result was almost three times better than in the case of the replanted soil with no additives (25.8 and 9.12 g, respectively) (Table 10). Furthermore, further increasing the amount of Carbomat Eco in the substrate to 30% and 45% of the volume increased the total mass of the side shoots of the trees to 30.0 and 41.3 g, respectively.

The fast growth of the side shoots of the apple trees in the treatments with the addition of Carbomat Eco to the replanted soil could have been the result of a large number of such shoots on the trees. In the treatments with the addition of Carbomat Eco lignite in the amounts of 30% and 45% of the substrate volume, the average number of shoots (6.7 and 6.5) was significantly higher than in the control combination and in the variant with the addition of biocarbon (Table 10). Mustafa and El-Shlasy [46] and Fatma et al. [47] also reported an increase in the number of side shoots under humic acid treatment.

Table 11 contains the results of the measurements of the growing power parameters of the aboveground and underground parts of apple trees. The researchers confirm the high efficiency of Carbomat Eco lignite as an additive in replanted soil. Both the weight of the main shoot and the length of the roots in combination with its use were significantly higher than in replanted soil with no additives. For example, the average root length of trees grown on soil with 30% Carbomat Eco (29.28 cm) was more than twice as long as in the combination without that additive (15.6 cm).

Table 11. Effect of substrate on the growing power parameters of the aboveground (cont.) and underground parts of the apple trees.

Treatments	Mass of the Main Shoot (g)	Root Mass (g)	Root Length (cm)
Agricultural soil	128.50 cd ¹	163.75 cd	22.96 b
Replanted soil	109.75 c	151.75 cd	15.06 a
Biocoal 5%	72.75 b	90.00 ab	12.13 a
Biocoal 10%	51.13 a	49.75 a	13.83 a
Biocoal 1 20%	69.75 ab	40.50 a	13.61 a
Carbomat Eco 15%	132.78 d	185.50 d	27.10 b
Carbomat Eco 30%	138.75 d	166.75 cd	29.28 b
Carbomat Eco 45%	134.50 d	143.10 cd	24.58 b

¹—The mean values marked with the same letters did not significantly differ at the $\alpha = 0.05$.

Figure 3 is a visual presentation of the growing power of the apple trees in different treatments. The well-developed root system of the trees allows the plant to be supplied with water and nutrients. The differences in the mass of the main shoot of the trees were smaller, ranging from approximately 20% (15% substrate volume in the soil) to 26% (30% volume). Increasing the amount of Carbomat Eco substrate in the soil from 15% to 45% did not significantly increase the mass of the main shoot of the apple trees or the length of their roots. However, both the mass of the main shoot of the apple trees and the length of their roots in the treatments with the addition of Carbomat Eco were significantly higher than those in the control variant with agricultural soil.

The high impact of Carbomat Eco lignite on the vegetative growth of apple trees can be confirmed by even a several-fold increase in the content of organic matter as a result of the supplementation of replanted soil with that additive, *inter alia* (Table 2). Treatment with the increased activity of soil microorganisms in such a substrate, measured by, e.g., soil respiration (Table 5), can significantly increase the number of available nutrients and

improve plant nutrition. In the experiment, in the leaves of the apple trees grown on replanted soil with the addition of Carbomat Eco in the amounts of 30% and 45% of the substrate volume, a significant increase in the content of nitrogen was observed (Table 6). As it is known, nitrogen is an element responsible for the vegetative growth of plants.

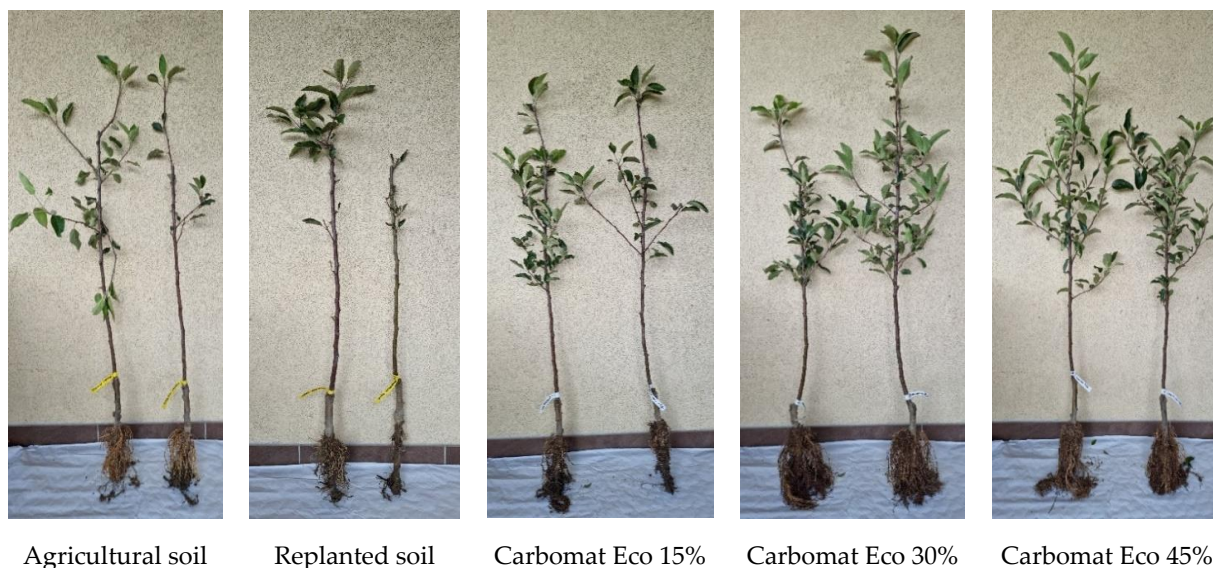


Figure 3. Effect of substrate on the growing power of apple trees.

Information about the effect of humic acids on root growth is inconclusive. The Schoebitz et al. [48] and Nunez et al. [49] experiments show that it did not significantly affect blueberry root growth. According to other authors, humic acids present in the soil additives can stimulate root growth, making it easier for plants to absorb nutrients from the soil [50–52]. After adding Carbomat Eco lignite to the replanted soil, regardless of its amount, the mass of the roots of the apple trees did not significantly change. In addition, adding biocarbon to the soil did not significantly change the growth parameters of the underground part of the apple trees. The length of the roots of the plants grown on such a substrate was similar to the length of the roots of the trees grown on the replanted soil with no additives, and the mass of the roots was even lower (Table 11). In the opinion of the authors, this may be due to the high content of phenolic compounds in the replanted soil with the addition of biocarbon. Their amount in treatments with 10% and especially 20% content of biocarbon in the substrate was almost twice as high as in the agricultural and replanted soil with no additives (Table 2). Phenolic compounds are formed from glycosides released as a result of decomposition by the microflora of root residues in the soil. The presence of remnants of apple tree roots in the soil increases the content of phenolic compounds in the soil up to seven times [53]. They are considered the biological reason for replant disease [42,54,55]. They slow down the development of soil microflora [56], which can cause deficits of nutrients in the soil, and limit the growth of apple rootstocks [57].

4. Conclusions

The previous method of using the soil significantly affected its physical, chemical and biological properties. The replanted soil used in the experiment was more acidic and less rich in organic matter than the agricultural soil. Its biological properties, measured by enzymatic activity and respiration, were also worse. Trees of similar height grew on the replanted soil; however, the growth rate of side shoots was significantly lower than those of the trees grown on the agricultural soil. The parameters of the leaves of the apple trees grown on the replanted and agricultural soils also differed to a great extent. The apple trees

grown on the replanted soil had a smaller leaf area, length and mass of leaves compared to those grown on the agricultural soil. The rate of photosynthesis was also lower.

By enriching the replanted soil with organic additives, its production properties could be significantly improved. Once the replanted soil was supplemented with Carbomat Eco lignite, its salinity decreased, while its respiration and enzymatic activity almost doubled. The high efficiency of using Carbomat Eco on replanted soil is confirmed by the measurement of the trees power of growth. The soil with Carbomat Eco produced taller apple trees with more side shoots, an increased mass of shoots and a larger leaf area than the replanted soil with no additives. Increasing the amount of Carbomat Eco in the soil did not significantly affect the vegetative growth of the apple trees. The biocarbon used in the experiment was less effective in terms of affecting the growth of the trees produced on the replanted soil than Carbomat Eco. Both organic additives significantly increased the rate of photosynthesis in the leaves of the trees grown on the replanted soil.

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