International Journal of Agricultural and Natural Sciences
E-ISSN: 2651-3617 18(3): 332-340, 2025
doi:10.5281/zenodo.18067382
Research Article

ASSESSMENT OF MICROALGAL APPLICATIONS ON SOIL PROPERTIES AT INCUBATION CONDITIONS

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(Received 2nd October 2025; accepted 28th November 2025)

ABSTRACT. Alternative soil improvement techniques have become important due to the adverse effects of intensive agricultural practices and various pollutants on soil properties. This study aims to evaluate the impact of *Spirulina platensis* and *Chlorella vulgaris* microalgae on specific soil qualities in a controlled environment. The microalgae used in the study were introduced into the soil at 1.5, 3, and 6 g kg^{-1}. The incubation time was designed to be the normal duration of 180 days. Soil samples were collected on Days 7, 15, 30, 60, 90, 120, and 180. The findings demonstrated that both microalgae species significantly decreased soil pH compared with the control (from 8.40 to 8.08; $p < 0.01$). Furthermore, soil electrical conductivity (EC) was markedly elevated relative to the control group, correlating with the escalating microalgae dosages and incubation duration (ranging from 268.06 µS cm^{-1} to 290.30 µS cm^{-1}). Soil organic matter (OM) and available phosphorus (AP) concentrations were markedly elevated in both microalgae species when treatment quantities escalated (from 2.64% to 2.94% for OM and from 5.88 to 7.24 mg kg^{-1} for AP). These results indicate that microalgae application represents a promising, low-cost, and environmentally friendly approach for improving the fertility of alkaline soils.

Keywords: *Microalgae, soil fertility, incubation, sustainable agriculture*

INTRODUCTION

Soil health and quality are crucial indicators of management efficiency and the long-term sustainability of meeting global food demand [1]. The decline of soil ecosystems is primarily attributed to intensive applications of chemical fertilizers and pesticides, excessive tillage practices that disrupt natural cycles, long-term monoculture farming systems, and the adverse effects of climate change on microbial diversity [2]. These factors lead to physical issues such as loosening soil structure and decreased overall stability. Furthermore, significant alterations occur in the soil's physical, chemical, and biological properties, including reduced organic matter (OM) content, nutrient loss due to leaching, and diminished microbial activity [3,4]. Environmentally friendly and sustainable biological practices are essential for improving soil quality and ensuring long-term productivity [5]. Among the most common biological approaches used to promote soil health and manage residues are rhizosphere microorganisms, bioinoculants, mycorrhizal fungi, biofertilizers, bioinoculants, and microalgae-based biostimulants [6]. In recent years, microalgae-based approaches have gained increasing attention due to their potential to improve soil fertility, carbon sequestration, and microbial activity while reducing dependency on synthetic fertilizers [7,8].

Microalgae are photosynthetic organisms comprising both prokaryotic and eukaryotic species, prevalent throughout several ecosystems [9]. Cyanobacteria (blue-green algae) and green algae differ in their physiological mechanisms. Cyanobacteria such as *Spirulina platensis* can fix atmospheric nitrogen and secrete extracellular polysaccharides (EPS) that improve soil aggregation and moisture retention. In contrast, green algae such as *Chlorella vulgaris* primarily contribute to carbon assimilation, nutrient recycling, and the production of plant

growth-promoting substances [10-12]. Microalgae enhance OM, facilitate the mineralization of macro and micronutrients, assimilate atmospheric CO_2, promote biological nitrogen fixation, stabilize soil, and stimulate microbial activity and plant growth via their phytohormones [13,14]. The exopolysaccharides produced by microalgae also contribute to carbon stabilization and soil aggregation, strengthening soil structure and long-term fertility [15,16]. Recent research has further highlighted their use as biofertilizers, demonstrating improved nutrient cycling, soil organic carbon accumulation, and crop performance under field and incubation conditions [17-21].

However, despite these promising findings, comparative studies assessing how different microalgal taxa (e.g., green algae vs. cyanobacteria) affect soil physicochemical properties under controlled incubation conditions remain limited. Most existing studies have focused on plant responses or short-term nutrient availability rather than soil-based transformations. This study, therefore, aimed to evaluate the effects of *Chlorella vulgaris* (a green alga) and *Spirulina platensis* (a cyanobacterium) on key soil properties, pH, electrical conductivity (EC), OM, and available phosphorus (AP) over a 180-day incubation period. The findings are expected to provide new insights into species-specific mechanisms and the potential of microalgae as bio-based soil amendments for sustainable agricultural management.

MATERIALS AND METHODS

Experimental Materials and Methodological Design

Trial Material and Design The soil material utilized in the incubation experiment was obtained at random from 0-30 cm soil depth at coordinates 38°45'30"N 35°26'51"E in the Kocasinan district of Kayseri province (Fig. 1). For the incubation experiment, the collected soil was spread out and air-dried before being sieved through a 4 mm steel sieve. To determine the initial general properties of the soil sample, the air-dried soil was passed through a 2 mm sieve to homogeneity, and then texture [22], pH, and electrical conductivity (EC) (1:2.5) [23], lime [24], OM [25], and AP [26] were analysed. All analyses were performed in analytical replicates (n = 3) and followed standard quality assurance and quality control (QA/QC) procedures to ensure data reliability and accuracy.

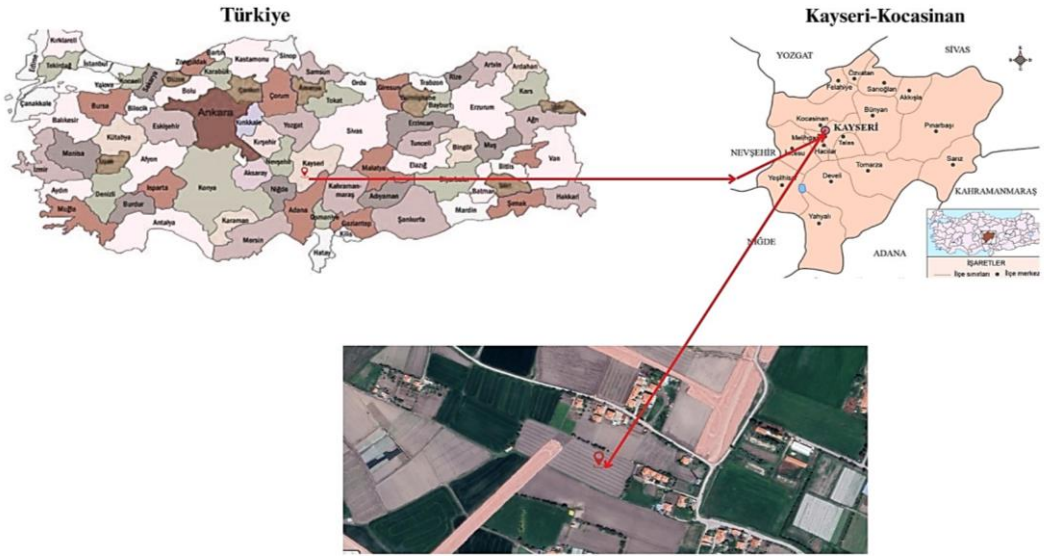

Fig. 1. *Illustration of the location from which the soil sample utilized in the study was collected*

Table 1. *Fundamental physical and chemical characteristics of the initial soil utilized in the experiment*

Texture	pH	EC	Lime	Organic Matter	Available Phosphorus
(%)		($\mu S\ cm^{-1}$)	(%)	(%)	($mg\ kg^{-1}$)
Tınlı Kum	8.4	268.06	2.17	2.64	5.88

According to the results of the studies, the initial soil was loamy sand in texture, slightly alkaline, salt-free, low in calcareous, with moderate OM and low AP. The microalgae *S.platensis* and *C.vulgaris* utilized in the experiment were provided as dry powder. The incubation study was conducted under controlled laboratory conditions using a completely randomized design with three replications, where each experimental unit consisted of 1 kg of air-dried soil placed in sealed incubation containers. The treatments in the experiment were the control group (no microalgae addition), *S.platensis* 1.5 g kg^{-1} (S1), 3 g kg^{-1} (S2), and 6 g kg^{-1} (S3), and *C.vulgaris* 1.5 g kg^{-1} (C1), 3 g kg^{-1} (C2), and 6 g kg^{-1} (C3) according to their dry weight calculations and mixed homogeneously into 1 kg of soil. During incubation, soil moisture was maintained at 60 % of field capacity by manual gravimetric adjustment, with containers weighed every two days and deionized water added to compensate for evaporation losses. The ambient temperature of the incubation room was kept constant at 23 ± 2 °C. The 180-day trial continued. Samples of soil were collected on days 7, 15, 30, 60, 90, 120, and 180. pH, EC, soil OM, and AP were determined in soil samples collected on the designated days. It should be noted that using soil from a single location (Kocasinan district, Kayseri) may limit the generalization of the findings to other soil types or climatic conditions; therefore, further studies using multiple soils with different characteristics are recommended.

Statistical Analysis

The experiment was conducted using a factorial, completely randomized design with three replications. The study's data were analyzed on a computer using the 'JMP 13.2.0' application. The Tukey Multiple Comparison Test was used to compare treatment mean values. Post-hoc comparisons were performed using Tukey's HSD test ($p<0.05$). All results are expressed as mean ± standard deviation (SD) [27].

RESULTS AND DISCUSSION

According to the findings, the interaction effect between application and incubation day on soil pH was statistically significant ($p \leq 0.01$). The soil pH values ranged from 8.40 ± 0.02 to 8.08 ± 0.01 (Table 2). The lowest pH value was observed in the C3 treatment on day 180, while the control group maintained the highest pH values throughout the incubation period. The overall pH decrease (approximately 3.8%) relative to the control is consistent with reports indicating that microalgae can acidify alkaline soils by releasing organic acids and stimulating microbial nitrification activity [28].

Table 2. *Mean soil pH values of different microalgae species as a function of incubation period*

Treatments	Incubation day					
	7	**15**	**30**	**60**	**120**	**180**
Control	8.40 ± 0.02 ^{a}	8.39 ± 0.02 ^{a}	8.37 ± 0.02 ^{abc}	8.38 ± 0.06 ^{ab}	8.40 ± 0.02 ^{a}	8.39 ± 0.01 ^{a}
C1	8.38 ± 0.04 ^{ab}	8.33 ± 0.01 $^{b-e}$	8.29 ± 0.01 $^{e-h}$	8.27 ± 0.01 $^{e-ı}$	8.25 ± 0.01 $^{f-j}$	8.18 ± 0.02 ^{klm}
C2	8.38 ± 0.03 ^{ab}	8.33 ± 0.01 $^{b-e}$	8.30 ± 0.01 $^{d-g}$	8.23 ± 0.02 $^{h-l}$	8.17 ± 0.02 ^{lm}	8.14 ± 0.02 ^{m}
C3	8.35 ± 0.02 $^{a-d}$	8.31 ± 0.01 ^{def}	8.28 ± 0.01 $^{e-h}$	8.22 ± 0.01 $^{ı-l}$	8.13 ± 0.01 ^{mn}	8.08 ± 0.01 ^{n}
S1	8.35 ± 0.01 $^{a-d}$	8.33 ± 0.03 $^{b-e}$	8.30 ± 0.03 $^{d-g}$	8.27 ± 0.03 $^{e-ı}$	8.24 ± 0.01 $^{g-k}$	8.20 ± 0.01 ^{jkl}
S2	8.36 ± 0.02 $^{a-d}$	8.31 ± 0.03 $^{c-f}$	8.27 ± 0.02 $^{e-ı}$	8.25 ± 0.01 $^{f-j}$	8.22 ± 0.01 ^{jkl}	8.18 ± 0.01 ^{klm}
S3	8.38 ± 0.03 ^{ab}	8.30 ± 0.02 $^{d-g}$	8.30 ± 0.01 $^{d-g}$	8.25 ± 0.01 $^{f-j}$	8.19 ± 0.01 ^{klm}	8.17 ± 0.01 ^{lm}
SE	0.006	0.007	0.007	0.012	0.018	0.020
Species	nsg					
Doses	**					
Species*Doses	nsg					
Time	**					
Species*Time	nsg					
Doses*Time	**					
Species*Doses*Time	nsg					

Means followed by the same letter are not significantly different at $p<0.05$ (Tukey test) (SE: standard errors).

All treatments and incubation day interactions statistically affected soil EC ($p \leq 0.01$). The EC values ranged from 268.06 ± 2.11 to 290.30 ± 3.28 µS cm^{-1} (Table 3). The highest EC value (290.30 µS cm^{-1}) was observed in the S3 treatment on day 180, whereas the lowest was recorded in the control group. Microalgae incorporation consistently increased soil EC compared to the control throughout the incubation period. The overall increase in EC (approximately 8%) indicates a higher concentration of soluble ions, likely due to the mineralization of microalgal biomass and the release of nutrients into the soil solution [29]. However, these values remain well below the salinity threshold for non-saline soils (<400 µS cm^{-1}), suggesting that the observed increase does not pose a salinization risk under the current conditions [30].

The treatment of *C.vulgaris* and *S.platensis* microalgae and incubation day interactions significantly affected soil OM levels ($p \leq 0.01$). The OM content ranged from 2.64 ± 0.05% to 2.94 ± 0.04% (Table 4). The highest OM concentration (2.94%) was recorded in the S3 treatment on day 180, while the lowest value (2.64%) was found in the control on day 7. All microalgae treatments showed a gradual increase in OM throughout the incubation period compared to the control. The average OM increase of approximately 11% relative to the control suggests that microalgae contributed organic inputs through biomass decomposition and microbial activity stimulation, which enhanced soil carbon accumulation. Although these differences were statistically significant, their magnitude is modest in agronomic terms, indicating a short-term improvement in soil carbon content. Over more extended periods, continuous application of microalgae could promote stable carbon sequestration and improve soil structure and fertility [31,32].

Table 3. *Mean soil EC values of different microalgae species as a function of incubation period*

Treatments	Incubation day					
	7	**15**	**30**	**60**	**120**	**180**
Control	268.06 ± 1.83 $^{e-m}$	267.00 ± 3.42 $^{e-m}$	268.23 ± 1.53 $^{e-m}$	267.73 ± 1.15 $^{e-m}$	267.66 ± 2.24 $^{e-m}$	270.20 ± 6.74 $^{d-k}$
C1	263.56 ± 3.53 $^{h-m}$	262.40 ± 5.80 $^{ı-m}$	261.73 ± 0.35 $^{j-m}$	268.16 ± 3.32 $^{e-m}$	272.00 ± 3.04 $^{c-j}$	275.36 ± 1.40 $^{b-g}$
C2	265.06 ± 3.46 $^{h-m}$	258.46 ± 3.56 ^{lm}	266.50 ± 1.31 $^{e-m}$	268.20 ± 2.31 $^{e-m}$	270.10 ± 2.98 $^{d-k}$	273.56 ± 1.55 $^{b-ı}$
C3	264.56 ± 2.32 $^{g-m}$	260.26 ± 4.55 ^{klm}	264.36 ± 1.60 $^{g-m}$	268.83 ± 5.05 $^{e-l}$	274.26 ± 3.20 $^{b-h}$	276.93 ± 1.47 $^{b-h}$
S1	267.00 ± 3.69 $^{e-m}$	264.73 ± 3.63 $^{g-m}$	265.10 ± 0.36 $^{f-m}$	276.00 ± 5.67 $^{b-f}$	283.03 ± 6.19 ^{abc}	289.36 ± 0.87 ^{a}
S2	264.56 ± 5.40 $^{g-m}$	259.60 ± 0.40 ^{klm}	264.70 ± 3.32 $^{g-m}$	274.00 ± 1.51 $^{b-h}$	284.03 ± 2.37 ^{ab}	288.40 ± 1.06 ^{a}
S3	263.13 ± 2.08 $^{h-m}$	257.30 ± 4.78 ^{m}	263.20 ± 1.04 $^{h-m}$	270.73 ± 2.70 $^{d-k}$	280.66 ± 5.88 $^{a-d}$	290.30 ± 3.47 ^{a}
SE	0.720	1.035	0.532	0.927	1.544	1.840
Species	*					
Doses	nsg					
Species*Doses	nsg					
Time	**					
Species*Time	nsg					
Doses*Time	*					
Species*Doses*Time	nsg					

Means followed by the same letter are not significantly different at p<0.05 (Tukey test) (SE: standard errors).

Table 4. *Mean soil OM values of different microalgae species as a function of incubation period*

Treatments	Incubation day					
	7	**15**	**30**	**60**	**120**	**180**
Control	2.64 ± 0.02 ^{qrs}	2.66 ± 0.01 $^{n-s}$	2.66 ± 0.03 $^{n-s}$	2.65 ± 0.02 $^{o-s}$	2.67 ± 0.01 $^{m-s}$	2.67 ± 0.01 $^{m-s}$
C1	2.64 ± 0.02 ^{qrs}	2.66 ± 0.02 $^{n-s}$	2.69 ± 0.01 $^{k-q}$	2.71 ± 0.02 $^{g-m}$	2.80 ± 0.01 ^{ef}	2.84 ± 0.01 ^{de}
C2	2.63 ± 0.02 ^{rs}	2.66 ± 0.01 $^{n-s}$	2.69 ± 0.01 $^{k-q}$	2.74 ± 0.01 ^{ghı}	2.84 ± 0.01 ^{de}	2.87 ± 0.01 ^{bcd}
C3	2.62 ± 0.01 ^{s}	2.68 ± 0.01 $^{l-r}$	2.70 ± 0.01 $^{ı-o}$	2.74 ± 0.02 ^{ghı}	2.85 ± 0.01 ^{cde}	2.92 ± 0.02 ^{ab}
S1	2.66 ± 0.02 $^{n-s}$	2.67 ± 0.01 $^{m-s}$	2.69 ± 0.01 $^{k-q}$	2.73 ± 0.01 $^{g-k}$	2.83 ± 0.01 ^{de}	2.86 ± 0.01 ^{cd}
S2	2.64 ± 0.02 ^{qrs}	2.68 ± 0.01 $^{l-r}$	2.71 ± 0.01 $^{g-m}$	2.75 ± 0.01 ^{fgh}	2.85 ± 0.01 ^{cde}	2.89 ± 0.01 ^{abc}
S3	2.64 ± 0.02 ^{qrs}	2.70 ± 0.01 $^{ı-o}$	2.72 ± 0.01 $^{g-l}$	2.76 ± 0.02 ^{fg}	2.87 ± 0.02 ^{bcd}	2.94 ± 0.02 ^{a}
SE	0.004	0.003	0.005	0.009	0.014	0.019
Species	**					
Doses	**					
Species*Doses	**					
Time	nsg					
Species*Time	nsg					
Doses*Time	**					
Species*Doses*Time	nsg					

Means followed by the same letter are not significantly different at p<0.05 (Tukey test) (SE: standard errors).

Treatments had a statistically significant effect on soil AP levels ($p \leq 0.01$), as did incubation day ($p \leq 0.05$). Soil AP values ranged from 5.88 ± 0.07 to 7.24 ± 0.09 mg kg^{-1} (Table 5). The highest AP concentration (7.24 mg kg^{-1}) was observed on day 180 in the C3 treatment, whereas the lowest value (5.88 mg kg^{-1}) occurred in the control group. All microalgae applications increased AP compared with the control, although the differences among treatments were not statistically significant. The overall increase of approximately 23% in AP relative to the control suggests that microalgae may enhance phosphorus solubility by exuding organic acids and

releasing phosphatase-like compounds. However, since this study did not measure enzymatic activities and microbial biomass, these mechanisms should be interpreted cautiously. Recent studies have reported similar findings, which observed improved phosphorus availability following the addition of soil microalgae [33,34].

Table 5. *Mean soil AP values of different microalgae species as a function of incubation period*

Treatments	Incubation day					
	7	**15**	**30**	**60**	**120**	**180**
K	5.88 ± 0.22 ^{b}	5.92 ± 0.20 ^{b}	5.89 ± 0.21 ^{b}	5.93 ± 0.20 ^{b}	5.92 ± 0.20 ^{b}	5.89 ± 0.23 ^{b}
C1	6.38 ± 0.67 ^{ab}	6.42 ± 0.62 ^{ab}	6.48 ± 0.53 ^{ab}	6.51 ± 0.54 ^{ab}	6.56 ± 0.57 ^{ab}	6.73 ± 0.35 ^{ab}
C2	6.56 ± 0.25 ^{ab}	6.78 ± 0.25 ^{ab}	6.81 ± 0.20 ^{ab}	6.88 ± 0.17 ^{ab}	6.86 ± 0.05 ^{ab}	6.99 ± 0.01 ^{ab}
C3	6.41 ± 0.41 ^{ab}	6.52 ± 0.49 ^{ab}	6.79 ± 0.29 ^{ab}	6.95 ± 0.08 ^{ab}	6.97 ± 0.04 ^{ab}	7.24 ± 0.11 ^{a}
S1	6.48 ± 0.52 ^{ab}	6.45 ± 0.51 ^{ab}	6.49 ± 0.53 ^{ab}	6.39 ± 0.51 ^{ab}	6.41 ± 0.52 ^{ab}	6.52 ± 0.38 ^{ab}
S2	6.58 ± 0.38 ^{ab}	6.78 ± 0.12 ^{ab}	6.86 ± 0.01 ^{ab}	6.92 ± 0.03 ^{ab}	6.94 ± 0.02 ^{ab}	6.96 ± 0.03 ^{ab}
S3	6.56 ± 0.29 ^{ab}	6.60 ± 0.35 ^{ab}	6.65 ± 0.35 ^{ab}	6.88 ± 0.10 ^{ab}	7.01 ± 0.10 ^{ab}	7.15 ± 0.20 ^{a}
SE	0.0917	0.0949	0.0943	0.0962	0.0997	0.1048
Species	nsg					
Doses	**					
Species*Doses	nsg					
Time	nsg					
Species*Time	nsg					
Doses*Time	nsg					
Species*Doses*Time	nsg					

Means followed by the same letter are not significantly different at $p<0.05$ (Tukey test) (SE: standard errors).

Numerous studies have demonstrated that microalgae applications improve soil physicochemical properties and stimulate plant growth by enhancing nutrient cycling, microbial activity, and soil aggregation [35-37]. The present findings support these general trends by showing that *C.vulgaris* and *S.platensis* influence key soil parameters through biochemical and microbial pathways rather than direct chemical effects. Microalgae act as living biostimulants that exude organic acids, amino acids, and EPS, collectively modifying the rhizosphere environment and enhancing nutrient bioavailability [39,40]. The decrease in soil pH observed in this study aligns with previous reports showing a 5-8% reduction in microalgae-amended soils [38]. This acidification is likely related to organic acid release and the oxidation of ammonium to nitrate during microbially mediated nitrification [34]. At the same time, the increase in EC, OM, and AP observed under microalgal treatments can be interpreted because of enhanced mineralization, microbial activation, and phosphorus solubilization processes [34, 38, 41]. Microalgal EPS plays a particularly critical role by binding cations, improving soil aggregation, and increasing water-holding capacity, thereby contributing to improved soil structure and fertility [11, 12, 42, 43]. The magnitude of these effects may vary depending on soil texture, buffering capacity, microalgae strain, and incubation duration. For instance, the alkaline soil used in this experiment (pH 8.4) may have accentuated acidification effects compared with neutral soils. At the same time, the 180-day incubation period allowed sufficient time for microbial decomposition and EPS accumulation [41]. In contrast, some cyanobacteria, such as *C.thermophila* or *Leptolyngbya* sp., have been reported to increase soil pH due to their alkaline metabolites and differing carbon assimilation pathways [21].

CONCLUSION

This study demonstrated that *C.vulgaris* and *S.platensis* microalgae can improve soil chemical properties under controlled conditions. As previous studies have suggested, these improvements are likely related to enhanced microbial activity, organic acid exudation, and gradual decomposition of algal biomass, which promote nutrient mineralization and increase the bioavailability of phosphorus and organic carbon. Both microalgae species slightly increased soil EC; however, the observed values remained within acceptable limits for non-saline soils. In soils already affected by salinity, continuous application may lead to salt accumulation over time; therefore, application rates should be adjusted according to initial EC values. Despite these promising outcomes, this study has several limitations, including using a single soil type, the absence of microbial biomass and enzyme activity measurements, and the relatively short incubation period. These factors may restrict the broader applicability of the results. From an applied perspective, the findings suggest that incorporating microalgae biomass into soil could be a foundation for developing biofertilizer formulations to improve soil fertility, enhance nutrient cycling, and increase carbon sequestration potential. Future research should validate these effects under greenhouse and field conditions, incorporating microbial and enzymatic analyses to better understand the mechanistic processes involved.

Overall, this work highlights the potential of *C.vulgaris* and *S.platensis* as sustainable, bio-based soil amendments that contribute to long-term soil health and environmentally friendly agricultural practices.

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