



Emerging contaminants in wastewater treatment: the effect of microplastics in an Integrated Fixed-film Activated Sludge (IFAS) Membrane BioReactor (MBR)

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ARTICLE INFO

Keywords:

Carbon footprint
Contaminants of emerging concern
Greenhouse gas emissions
Membrane technology
Wastewater Treatment Directive

ABSTRACT

Microplastic (MP) pollution is increasingly recognized as one of the most urgent global environmental challenges. Despite the exponential growth in plastic production, only 9 % of plastic waste is currently recycled, contributing significantly to environmental pollution. Wastewater treatment technologies need to be evaluated for their effectiveness, also for emerging contaminants such as MP. This study features a pilot Integrated Fixed Film Activated Sludge (IFAS) reactor followed by two parallel lines: Line I, incorporating a membrane bioreactor (MBR), and Line II, consisting of secondary sedimentation and tertiary ultrafiltration. The main objectives were to assess the IFAS-MBR system's performance in terms of carbon and nutrient removal, greenhouse gas emissions and MP removal. The results demonstrated excellent removal efficiencies for carbon (97 % and 98 % in Line I and II, respectively), nitrogen (76 % and 82 % in Line I and II, respectively), and a comparable N₂O emission factor (0.19 % of the influent total nitrogen in both lines). In terms of MPs, starting from an influent concentration of 10 mg L⁻¹ polyethylene, effluent MPs concentrations were reduced to approximately 0.02 mg L⁻¹, achieving a 99.8 % removal rate in both lines. These findings highlight the potential of integrated IFAS-MBR systems in addressing multiple wastewater treatment targets, including emerging contaminants.

1. Introduction

Emerging pollutants, including microplastics (MPs), are increasingly recognized as a significant environmental concern (Christou et al., 2024). Currently, over seven billion tons of plastic waste are generated, with only 9 % recycled. The remaining waste is either landfilled, incinerated or released into the environment (United Nations Environment Programme UNEP, 2021). Water systems serve as major conduits for MPs, as they accumulate from domestic, industrial and urban runoff sources (Strokal et al., 2023). Consequently, wastewater treatment plants (WWTPs) have been identified both as collectors and potential emitters of MPs (Wu et al., 2024). It has been estimated that WWTP receive approximately 1.05×10^5 to 1.47×10^{13} particles of MPs daily (Hu et al., 2024; Kruglova et al., 2022).

Although conventional WWTPs were not originally designed to target MPs, several studies demonstrate removal efficiencies exceeding

90 %, primarily due to the capture of MPs within sludge matrices (Rashid et al., 2025; Talvitie et al., 2017; Wu et al., 2024). However, sludge reuse practices raise concerns regarding the reintroduction of MPs into the environment (Foglia et al., 2024). In response to this growing issue, the European Commission recently introduced the Directive on Urban Wastewater Treatment (Directive 2024/3019), emphasising water reuse practices, achieving 100 % energy neutrality by 2040, nutrient recovery and monitoring for emerging pollutants. Given this regulatory evolution, there is a growing necessity to evaluate innovative technologies capable of simultaneously achieving high efficiencies in nutrient and micropollutant removal. Several studies have been conducted to understand the best available technology in “conventional” wastewater pollutants such as phosphorous and nitrogen. In particular, membrane bioreactors (MBRs), which combine biological activity with membrane filtration, have demonstrated high performance but are challenged by membrane fouling (Y. Liu et al., 2025; Z. Liu et al.,

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2025; Yoshino et al., 2024). Combining MBR with Integrated Fixed Film Activated Sludge (IFAS) systems has proven particularly promising in reducing membrane fouling and enhancing nutrient removal due to the presence of attached biomass (Lan et al., 2024; Mannina et al., 2020). However, there is still a lack of literature exploring how IFAS systems perform in terms of MPs removal. According to the literature, a significant portion (50–85 %) of MPs entering the wastewater system is removed in preliminary/primary treatment, 8–35 % is removed by secondary treatment, and only 2–8 % is removed by tertiary treatment (Komorowska-Kaufman and Marciniak, 2024). In particular, advanced technologies in tertiary treatment differ in their removal efficiency due to MPs size of the particles. In fact, larger MPs are already removed during primary treatments. In contrast, lighter and smaller MPs (diameter > 0.05 mm), such as PE, remain suspended and can only be eliminated through tertiary treatments (Zoccali et al., 2025). Among the available technologies, membrane bioreactor (MBR) systems are the most effective for MP removal, with reported efficiencies of up to 99 % (Cavazzoli et al., 2025; Sun and Jiang, 2025; Zoccali et al., 2025). Critical challenges persist, such as MPs acting as vectors for other emerging contaminants, potential inhibition of key biological processes (e.g., nitrification/denitrification), and promoting membrane fouling (Adeel et al., 2024). Due to their intrinsic properties, such as hydrophobicity and a high surface area-to-volume ratio, MPs can adsorb a wide range of pollutants. Evidence suggests that they may particularly affect secondary treatment processes by interacting with activated sludge microorganisms, leading to variations in greenhouse gas emissions, changes in nitrification and denitrification efficiency, and even restructuring of the microbial community within the system (Sun and Jiang, 2025; He et al., 2024). Importantly, MPs can serve as habitats for microorganisms: bacteria initially colonize their surfaces, secrete extracellular polymeric substances (EPS), and develop complex biofilm structures called “plastispheres” (Sun and Jiang, 2025).

Furthermore, IFAS systems due to the biofilm can achieve higher removal efficiencies than conventional plants, entrapping MPs (Ma et al., 2024). Among the available advanced technologies, membrane bioreactor (MBR) systems stand out as the most effective for MP removal, with reported efficiencies up to 99 % (Sun and Jiang, 2025; Di Bella et al., 2022).

However, standardized protocols for MPs extraction and quantification remain lacking, complicating data comparison across studies (Komorowska-Kaufman and Marciniak, 2024; Yaseen et al., 2022).

This study aims to contribute to the research gaps described above by valuating the performance of two IFAS-membrane systems. The IFAS biological reactor is coupled with a side-stream MBR in Line I and a settle-ultrafiltration (CAS-UF) system in Line II, operated in parallel. The two systems are compared based on their efficiency in carbon and nutrient removal, N₂O emissions, carbon footprint and finally, MPs removal efficiency. This study aims to provide insights into the efficiency of an IFAS-MBR system in MPs removal. It will also offer a complete overview by comparing MBR and CAS-UF systems.

2. Materials and methods

2.1. Pilot plant configuration

The monitoring campaign was conducted at the University of Palermo's Resource Recovery Facility (Mannina et al., 2021). The pilot plant collects wastewater from the university's canteen and dormitories, ensuring a sewage composition similar to that of domestic wastewater. The pilot plant configuration features a biological reactor (225 L) equipped with an IFAS system utilizing carriers (40 L, 0.95 g cm⁻³ density and 500 m² m⁻³ specific surface area), which combines suspended and attached biomass. After the IFAS reactor, Line I sludge is pumped into an MBR side-stream reactor (48 L) equipped with a hollow fibre ultrafiltration membrane (PURON® Koch Membrane Systems, 0.03 µm porosity and 1.4 m² surface area). Parallely, in Line II, sludge is

settled in a secondary sedimentation tank (46 L), after which the clarified water is treated through tertiary UF using a hollow fibre membrane identical to the one used for Line I (Fig. 1). Both membranes were operated in a cycle of 10 min (9 min of filtration and 1 min of backwash); permeates were collected in a tank used for backwash. The sewage enters the plant with a flow rate of 36 L/h and is pumped at a flow rate of 82 L/h to the MBR and the settler. Both return activated sludge lines go to an oxygen depletion reactor (ODR) for deoxygenation, with a flow rate of 63.2 L/h, resulting in an outlet flow rate of 18 L/h for each line.

2.2. Operational parameters

The system was operated at a fixed mixed liquor suspended solids concentration of 6.1 ± 0.4 g L⁻¹ while the attached biomass suspended solids concentration was 1.04 ± 0.02 g L⁻¹. The average sludge retention time was equal to 8.1 ± 0.2 days, while the hydraulic retention time was maintained at 8 h. The average food to microorganisms ratio was equal to 0.16 ± 0.12 kgBOD kgSS⁻¹ day⁻¹. Dissolved oxygen concentration in the biological reactor was approximately 2.6 ± 0.7 mg L⁻¹. The average and standard deviation (SD) characteristics of the influent wastewater are reported in Table 1. Wax powder (DEUREX E 0920 M, Germany) was dosed in the biological reactor to achieve a 10 mg PE L⁻¹ influent concentration, as reported in the literature (Corpuz et al., 2024).

2.3. Analytical methods

The system was monitored twice a week by sampling as shown in Fig. 1. Liquid samples were collected to monitor chemical oxygen demand (COD) and biochemical oxygen demand (BOD₅), ammonia (NH₄-N), nitrates (NO₃-N), nitrites (NO₂-N), total nitrogen (TN), orthophosphate (PO₄-P), and total and volatile suspended solids (TSS and VSS, respectively) according to standard methods (American Public Health Association, 2023). Each chemical analysis was conducted in duplicate. The carbon to nitrogen ratio (C/N) is calculated as the ratio between the influent sCOD and influent TN.

Dissolved and gaseous N₂O concentrations were measured in duplicate using a procedure reported in the literature, adopting a Gas Chromatograph (Agilent 8860) equipped with an electron capture detector (Mannina et al., 2018). N₂O sampling was conducted by taking samples twice a week, both for gaseous and dissolved forms, following the procedure described in the literature (Mannina et al., 2018). The sampling involved the biological reactor, MBR, settler and UF unit in order to assess the total plant-wide N₂O emission comprehensively. Each analysis was carried out in duplicate. Gaseous N₂O samples were directly withdrawn from the reactor's headspace thanks to the covers and sampling points installed. The stripped N₂O dissolved in the mixed liquor and effluent were sampled and centrifuged at 8000 rpm for 5 min. 70 mL of the supernatant was stored in glass bottles (total volume, 125 mL) and acidified with 1 mL of 2 N H₂SO₄. The bottles were kept mixing for 24 h. Afterwards, the bottle's headspace was sampled to collect the dissolved N₂O samples. Gaseous and dissolved N₂O concentrations are used to calculate the emission factor (EF_{N₂O}), according to Eq. (1), to assess the comprehensive N₂O emission (Tsuneda et al., 2005):

$$EF_{N_2O} = \frac{N_2O - N_g / HRT_{HS} + N_2O - N_d / HRT}{TN} \quad (1)$$

where N₂O-N_g and N₂O-N_d are the gaseous and dissolved nitrous oxide concentrations, respectively, HRT is the pilot-plant hydraulic retention time, HRT_{HS} is the retention time in the tank headspace, and TN is the total nitrogen concentration in the influent flow.

The carbon footprint was calculated as the sum of direct, indirect, and derived emissions (Boiocchi et al., 2023). Direct emissions have been calculated by quantifying the equivalent CO₂ produced through organic carbon oxidation (CO_{2,OrgOx}) (Eq. (2)), endogenous respiration

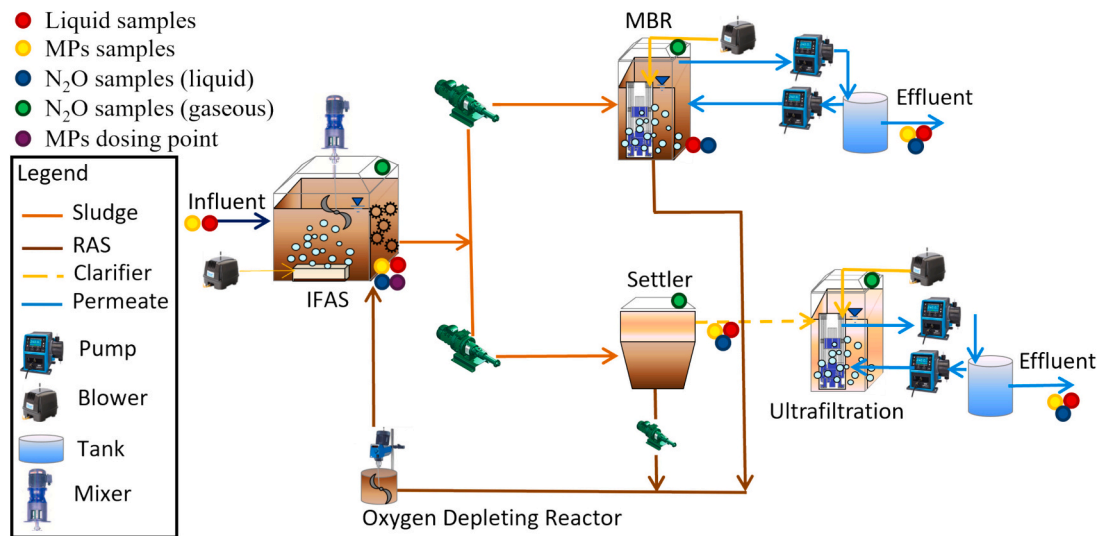


Fig. 1. Plant configuration scheme. Sampling points are highlighted with different colours for physical-chemical analysis (red), microplastics analysis (yellow), N₂O dissolved (blue), and N₂O gaseous (green). Purple shows the MPs dosing points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Influent wastewater average characteristics and standard deviation (SD).

Parameter	Symbol	Units	Average	SD
Total chemical oxygen demand	tCOD	mg L ⁻¹	1532	429
Soluble chemical oxygen demand	sCOD	mg L ⁻¹	272	103
Biochemical oxygen demand	BOD	mgO ₂ L ⁻¹	448	61
Ammonium	NH ₄ ⁺	mgN L ⁻¹	28	15
Total Nitrogen	TN	mgN L ⁻¹	47	16
Orthophosphate	PO ₄ ³⁻	mg L ⁻¹	5	3
Total suspended solids	TSS	g L ⁻¹	0.7	0.3
Volatile suspended solids	VSS	% of TSS	50	1
Microplastics (polyethylene - PE)	MPs	mg L ⁻¹	0.3	0.1

(CO_{2,Endog}) (Eq. (3)), and the N₂O emitted during nitrogen biological removal (CO_{2,N2O}) (Eq. (4)). Indirect emissions were obtained by quantifying the equivalent CO₂ from water line energy consumption (CO_{2eq,En}) (Eq. (5)) and the mass of wasted sludge (CO_{2eq,Sludge}) (Eq. (6)). Derived emissions were calculated by quantifying the equivalent CO₂ from effluent pollutants, especially BOD (CO_{2eq,effBOD}) (Eq. (7)) and dissolved N₂O (CO_{2eq,effN2O}) (Eq. (8)).

$$CO_{2,OrgOx} = FC_S \cdot r_{O_2} \quad (\text{kg CO}_2 \text{ d}^{-1}) \quad (2)$$

where FC_S is the conversion factor (kg CO₂ per kg O₂ consumed) and r_{O₂} is the daily oxygen consumption.

$$CO_{2,Endog} = FC_{End} \cdot m_{VSS} \quad (\text{kg CO}_2 \text{ d}^{-1}) \quad (3)$$

where FC_{End} is the conversion factor (kg CO₂ per kg VSS) and m_{VSS} is the term related to the volatile suspended solids mass (kg).

$$CO_{2,N2O} = Q_g \cdot C_{g,N2O} \cdot GWP_{N2O} \quad (\text{kg CO}_{2eq} \text{ d}^{-1}) \quad (4)$$

where Q_g is the gas flow rate (m³ d⁻¹), C_{g,N2O} is the concentration of N₂O in the gas phase (kg m⁻³) and GWP_{N2O} is the global warming potential of N₂O (298, IPCC).

$$CO_{2eq,En} = E_n \cdot FC_{En} \quad (5)$$

where E_n is the electricity consumption (kWh d⁻¹) and FC_{En} is the conversion factor (0.252 kg CO_{2eq} kWh⁻¹, EEA).

$$CO_{2eq,Sludge} = M_{Sludge} \cdot FC_{Sludge} \quad (6)$$

where M_{Sludge} is the daily wasted sludge mass (tons d⁻¹) and FC_{Sludge} is the conversion factor for sludge management (714.74 kg CO_{2eq} ton⁻¹), including treatment, transportation, and disposal.

$$CO_{2eq,effBOD} = M_{BOD} \cdot FC_{BOD} \quad (7)$$

where M_{BOD} is the BOD discharged in the effluent (kg d⁻¹) and FC_{BOD} is the conversion factor (0.96 kg CO_{2eq} kgBOD⁻¹).

$$CO_{2eq,effN2O} = Q_w \cdot C_{l,N2O} \cdot GWP_{N2O} \quad (8)$$

where Q_w is the treated water flow (m³ d⁻¹) and C_{l,N2O} is the average N₂O concentration in the effluent (kg m⁻³).

2.4. Microplastics extraction and analysis protocol

The MPs' extraction and analysis protocol followed the methodology proposed by the literature (Giannattasio et al., 2024). The detection protocol is divided into three steps, as shown in Fig. 2: filtration, solid-liquid separation and qualitative/quantitative analysis. Around 200 mL of samples are filtered using a Buchner apparatus equipped with a nitrocellulose filter (0.45 μm pore size, 50 mm diameter). Following filtration, the filter is carefully removed and allowed to air dry for 24 to 48 h. To minimise the risk of external contamination, the filter is placed in two Petri dishes or between two sheets of blotting paper (e.g., Scottex) for protection. Once fully dried, the filter is transferred into a 50 mL Falcon tube, which is then filled with 99.9 % acetone. The nitrocellulose filter is dissolved in acetone at room temperature. Following complete dissolution, the sample is centrifuged at 9500 rpm for 10 min. After centrifugation, the supernatant is gently removed, and the sample is refilled with acetone before being subjected to a second round of centrifugation. Finally, the microplastic particles, which settle to the bottom of the Falcon tube, are left to air-dry at room temperature. The tube is covered with blotting paper to prevent contamination during the drying process. The settled microplastics are transferred into a 4 mL vial, to which 700 μL of 1,1,2,2-tetrachloroethane-*d*₂ (TCE-*d*₂) (99.5 %) is added. The resulting mixture is then heated at 80 °C for 24 h to ensure the dissolution of TCE-*d*₂-soluble polymers. Afterwards, 9.7 μL of mesitylene (99.9 %) are added to the TCE-*d*₂ solution (C = 0.1 M) as an internal standard. The resulting solution is carefully transferred into a nuclear magnetic resonance (NMR) analysis tube. NMR spectra were recorded at 80 °C on a Bruker AVANCE 400 MHz spectrometer (for ¹H

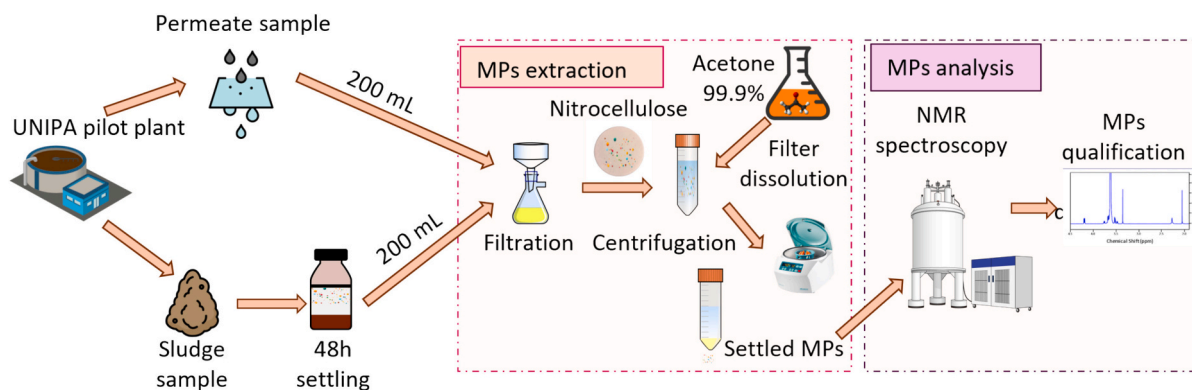


Fig. 2. Schematic view of the MPs extraction and analysis protocol.

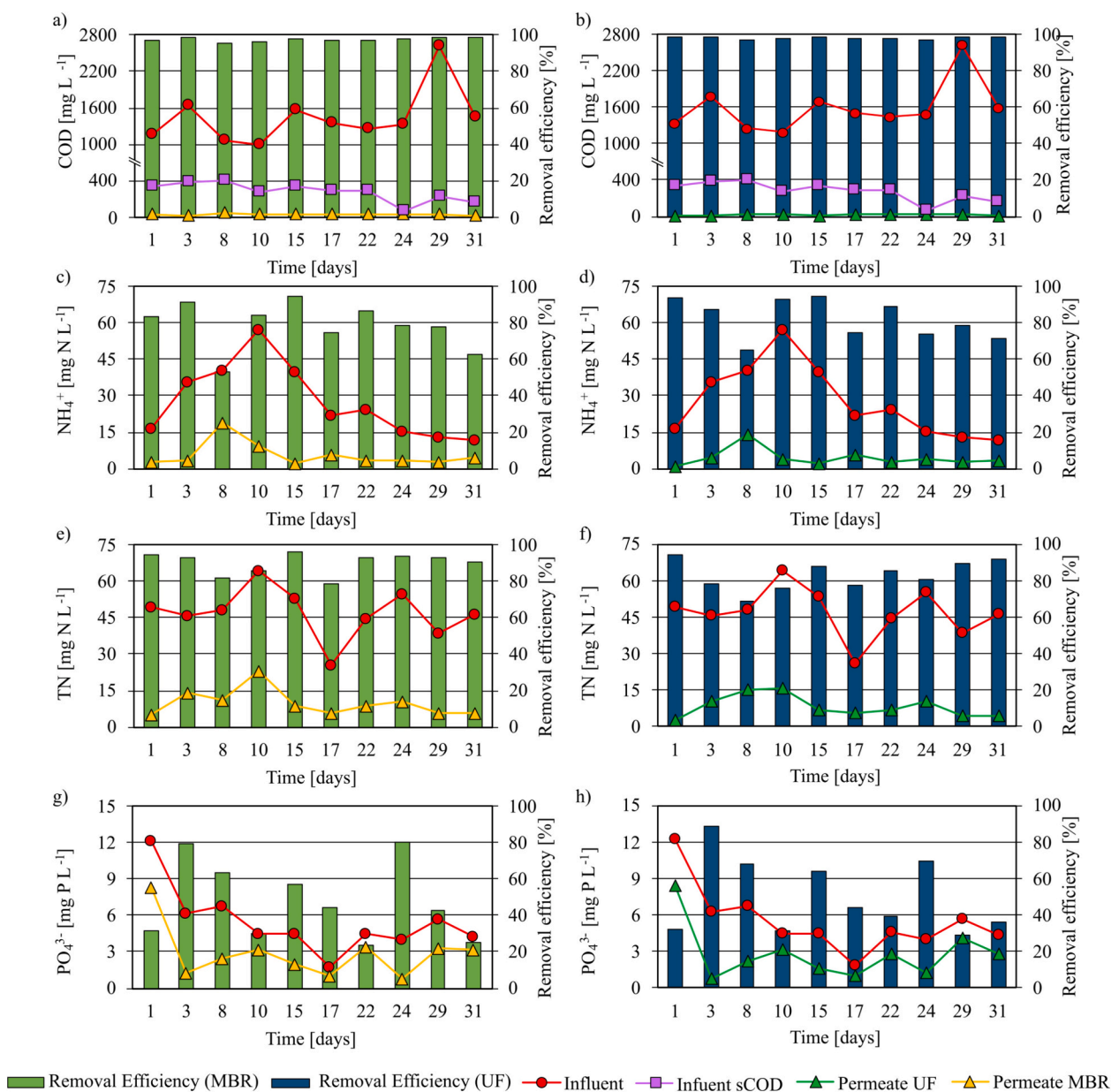


Fig. 3. Concentrations and removal efficiencies for COD (a, b), ammonium NH_4^+ (c, d), total nitrogen TN (e, f) and phosphate PO_4^{3-} (g, h) for MBR and UF line, respectively.

NMR experiments, the following instrumental parameters were used: $d1 = 2.0$ s; $ns = 256$) and all chemical shifts are reported in parts per million (ppm, δ). Chemical shifts are reported, calibrating the residual solvent peak of TCE- d_2 at 5.80 ppm. As positive control experiments, the recovery efficiencies of PE were determined for 5 samples of deionised water (2 L) seeded with PE, applying the above-described procedure: the recovery efficiency was $>99\%$. LOQ (0.001 mg L^{-1}) was calculated as previously reported in the literature PE concentrations [PE] were determined with the following equation (Giannattasio et al., 2024):

$$[\text{PE}] \left(\frac{\text{mg}}{\text{L}} \right) = \frac{\left(\frac{A_{\text{CH}_2\text{PE}}}{2} \right) - mn_{\text{IS}} \cdot MW_{\text{CH}_2}}{\left(\frac{A_{\text{CH}_2\text{S}}}{3} \right) V_{\text{SW}}} \quad (11)$$

where $A_{\text{CH}_2\text{PE}}$ is the area of the ^1H NMR signal of PE methylene groups (signal at 1.14 ppm), $A_{\text{CH}_2\text{S}}$ is the area of the ^1H NMR signal of internal standard methine groups (mesitylene, signal at 6.63 ppm), mn_{IS} is the $mmol$ content of the internal standard, MW_{CH_2} is the molecular weight of the CH_2 group, V_{SW} is the volume of sampled water. Representative NMR spectra used for PE determination are reported in the Supplementary materials.

3. Results and discussion

3.1. Carbon and nutrient removal

Fig. 3 shows carbon and nutrient concentration trends observed in the influent and effluent of both Line I (MBR) and Line II (UF) configurations. Both lines demonstrated high carbon removal efficiencies, with COD removal reaching 97.1 % in Line I and 98 % in Line II, corresponding to final effluent COD concentrations of 28.8 mg L^{-1} and 35.1 mg L^{-1} , respectively (Fig. 3a,b). These outcomes align closely with previous studies on pilot-scale IFAS-MBR systems under similar operating conditions (Mannina et al., 2020; Wang et al., 2021). In terms of nitrogen removal, Fig. 3c shows that Line I achieved 73 % biological ammonium ($\text{NH}_4^+\text{-N}$) removal, with a total removal of 78 %, while Line II showed 78 % biological and 84 % total removal efficiency. The effluent concentrations of $\text{NH}_4^+\text{-N}$ were 5.5 mg L^{-1} for Line I and 8.4 mg L^{-1} for Line II, indicating effective nitrification across both systems. Total nitrogen (TN) removal efficiencies were 90 % for Line I and 83 % for Line II, indicating good efficiency in both lines. Influent TN concentrations averaged around 47 mg L^{-1} , while the effluent concentrations reached 10 mg L^{-1} in Line I and 8 mg L^{-1} in Line II. These results demonstrate compliance with the stringent effluent requirements set by the new Urban Wastewater Treatment Directive, particularly for Line II. Phosphorus removal (Fig. 3g, h), although not targeted explicitly through enhanced biological phosphorus removal strategies, was moderate in both systems. The MBR line achieved a 48 % removal, while the CAS-UF line achieved a 50 % removal, highlighting the potential for

partial phosphorus mitigation even under non-optimized conditions (Y. Liu et al., 2025; Z. Liu et al., 2025).

Table 2 summarises the influent characterization and removal efficiencies of similar IFAS systems reported in the literature. Similar COD results with an IFAS-MBR system can be found in Mannina et al. (2020). According to this study, a pilot plant with a similar influent COD concentration (1340 mg L^{-1}) achieved 97 % COD removal. On the other hand, IFAS systems with a settler generally demonstrate lower carbon removal efficiencies, reaching up to 94 % (Corsino et al., 2024; Dolatshah et al., 2024). Nitrogen removal efficiency aligns with the literature, demonstrating high performance in both nitrification and denitrification rates (Lan et al., 2024). Despite the system operating in continuous aeration mode, the biofilm on the carrier's surface ensured anoxic/anaerobic conditions, achieving high denitrification rates in both lines (Xu et al., 2021). Furthermore, although the introduction of microplastics at 10 mg PE L^{-1} could have disturbed the biological activity, no significant worsening effects on nitrification or denitrification efficiencies were observed. These results are in contrast with the literature. In a study conducted by Wu et al. (2024), increasing the concentration of PE from 100 to $1000 \mu\text{g L}^{-1}$ led to a decrease in denitrification efficiency, from 90 % to 50 %. It was observed that PE, in particular, induces oxidative stress due to the increased production of reactive oxygen species, which cause cell death and alter cellular metabolism, interfering with gene expression. In particular, it can inhibit or enhance the expression of genes responsible for nitrification and/or denitrification, increasing N_2O emission (He et al., 2024; Wu et al., 2024).

Zhou et al. (2022) observed a reduction in nitrogen removal efficiency, dropping from 97 % to 72 % when the concentration of MPs exceeded 1 mg L^{-1} . Moreover, in the study by Lee et al. (2022), no significant effect on nitrification rate was observed. The genomic analysis was conducted on the enzymes related to ammonia oxidation, and it was observed that MPs slightly increased the expression of the *amoA* gene, suggesting that MPs could influence the nitrification process. These results suggest that carriers may mitigate MPs-induced biological stress, considering that tenfold higher MP concentrations were used in this work without any noticeable deterioration in performance.

In terms of phosphorous removal, despite the overall good PO_4^{3-} removal efficiency achieved, phosphorus removal remains a critical issue in WWTPs, especially in view of the stringent requirements of the new EU Directive 3019/, 2024. PO_4^{3-} chemical precipitation can be considered as an option to increase removal efficiency without changing the plant configuration, achieving high efficiency (90–95 %), but requires high operational costs. From a circular economy perspective, another cost-effective solution is adsorption with adsorbent materials such as biochar, which ensures high P removal ($>90\%$) and can be directly used as a slow-release fertilizer (Zheng et al., 2023).

3.2. Nitrous oxide emission and carbon footprint

The N_2O emission factor (EF) is reported in Fig. 4 for both lines. The EFs were remarkably similar between the two lines, averaging $0.19 \pm$

Table 2
Average influent concentrations and removal efficiencies for IFAS systems.

Configuration	Influent COD [mg L^{-1}]	Influent NH_4^+ [mg N L^{-1}]	Influent TN [mg N L^{-1}]	η_{COD} [%]	η_{NH_4} [%]	η_{TN} [%]	$\eta_{\text{N den.}}$ [%]	$\eta_{\text{N nitr}}$ [%]	η_{PO_4} [%]	Reference
IFAS-MBR	310 ± 159	25 ± 14	38 ± 10	95.3	91.8	78.1	–	–	93.7	Wang et al. (2021)
IFAS-MBR	90 ± 30.6	20 ± 7	24 ± 9	88.5	98.7	71.3	–	–	–	Yoshino et al. (2024)
IFAS-MBR	1340 ± 565	100 ± 43	–	97	70	58	48	69	60	Mannina et al. (2020)
IFAS-MBR	1532 ± 439	28 ± 15	47 ± 10	97	78	90	49	73	47.7	This study
IFAS w settler	425 ± 36	49 ± 20	–	90	–	65	12	30	60	Corsino et al. (2024)
IFAS w settler	1015 ± 107	189 ± 39	197 ± 34	94.4	–	45	–	–	–	Dolatshah et al. (2024)
IFAS w settler	1532 ± 439	28 ± 15	47 ± 10	98	82	81	62	78	50.2	This study

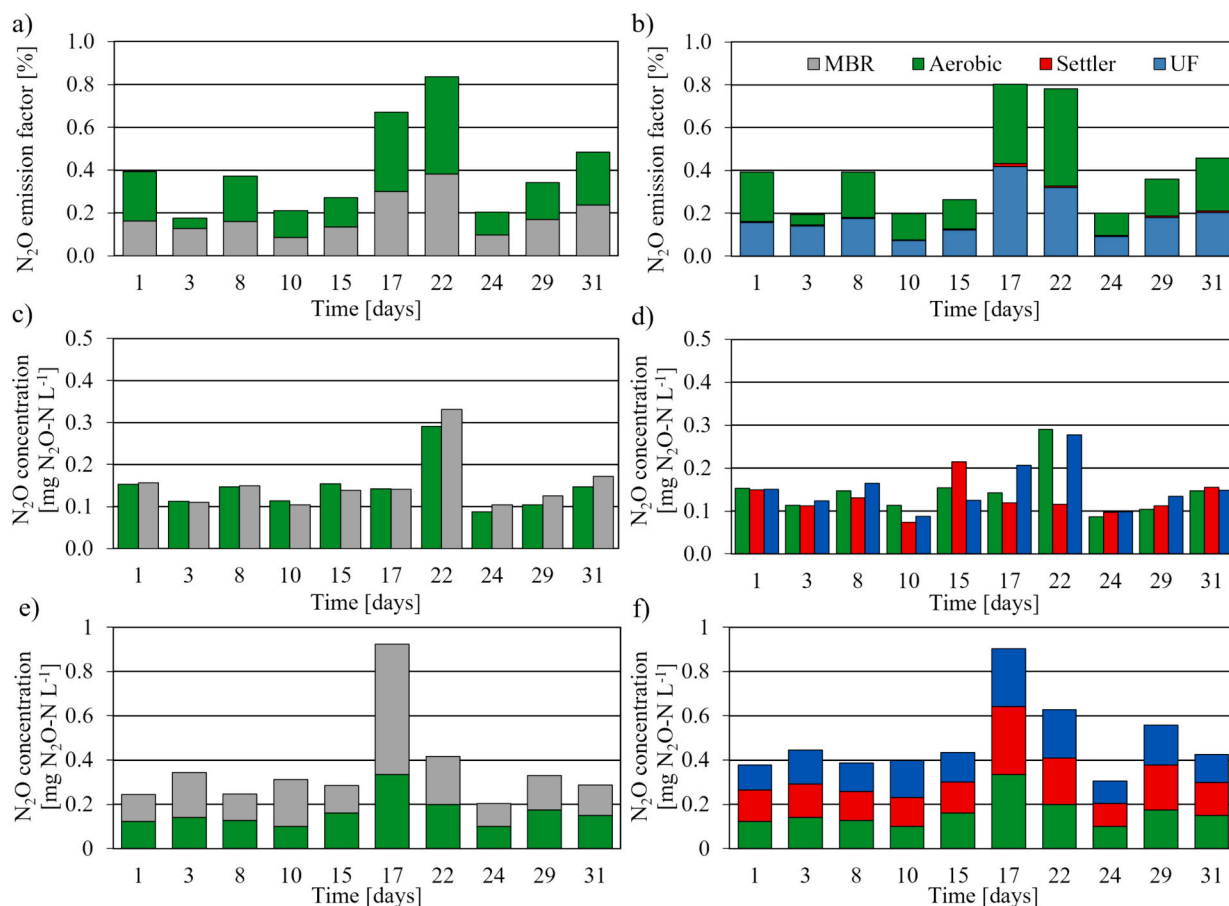


Fig. 4. N₂O emission factor (a, b), N₂O gaseous (c, d) and dissolved concentrations (e, f) measured for all the reactors of MBR and UF lines, respectively.

0.09 % of the influent total nitrogen. These results are consistent with previous research, which indicates that IFAS systems promote more stable nitrogen removal processes with lower associated N₂O emissions compared to conventional activated sludge systems. The biofilm carriers in the IFAS reactor likely contributed to this mitigation by creating microenvironments conducive to complete denitrification, thereby reducing intermediate accumulation of N₂O. These results are consistent with the findings of He et al. (2023), who compared a system containing only sludge flocs with an IFAS system. The study showed that N₂O emission factors in the IFAS system were consistently lower than those in the sludge flocs system, regardless of the dissolved oxygen concentration. A similar IFAS system was monitored by Mannina et al. (2017), who reported comparable results, with emission factors ranging from 0.13 % to 0.73 % of the total influent nitrogen. Moreover, Mannina et al.

(2018) compared an aerated reactor with an IFAS reactor and demonstrated a reduction in the emission factor from 3.5 % to 0.5 %. In Fig. 4c–f, N₂O concentrations measured in both lines are reported. The average aerobic gaseous and dissolved N₂O concentrations were 0.15 ± 0.06 mg N₂O-N L⁻¹ and 0.16 ± 0.07 mg N₂O-N L⁻¹, respectively. Although the MBR and UF maintained similar N₂O concentrations in the gaseous phase (0.15 ± 0.07 mg N₂O-N L⁻¹ for MBR and 0.15 ± 0.06 mg N₂O-N L⁻¹ for UF), the MBR exhibited a higher dissolved N₂O concentration (0.19 ± 0.14 mg N₂O-N L⁻¹). However, the high variability, indicated by the large standard deviation, was mainly due to the N₂O concentration peak observed on day 17 (0.59 mg N₂O-N L⁻¹). The efficiency of the IFAS system is further demonstrated by its performance in total nitrogen removal (Fig. 3). Fig. 5 illustrates the distribution of the effluent nitrogen fractions. For both lines, the denitrified N accounted

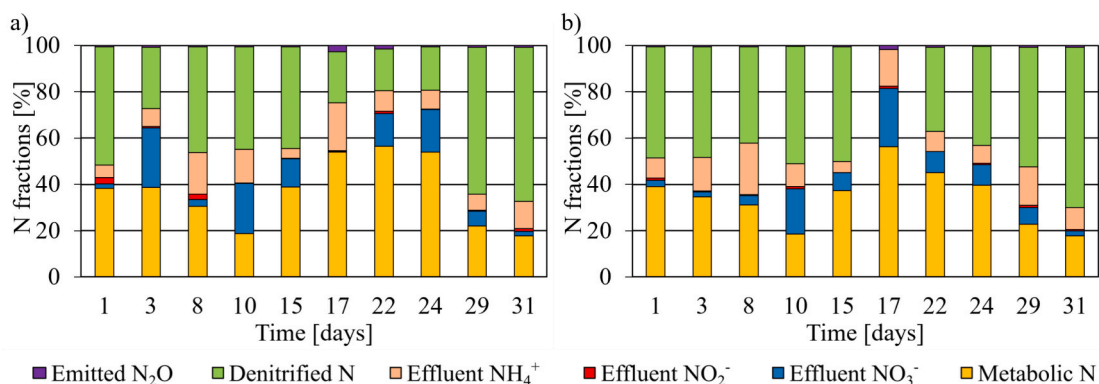


Fig. 5. Nitrogen effluent fractions for MBR (a) and UF (b) lines.

for the highest share, 40 % and 44 % for the MBR and UF Lines, respectively. The metabolic nitrogen share accounted for 37 % and 34 % for the MBR and UF line, respectively. The higher N_2O EF on days 17 and 22 (0.65 % and 0.83 % for MBR line and 0.80 % and 0.77 % for UF line, respectively) are related to the higher metabolic nitrogen fractions (around 53 %) observed (Fig. 5). Indeed, a sudden drop in influent ammonium concentration occurred on day 17, with similar values maintained until day 22. The sudden C/N shift (from 6 g sCOD gTN⁻¹ to 9 g sCOD gTN⁻¹) highly affected N_2O EF, mainly because of incomplete nitrification in the MBR line and incomplete nitrification/denitrification in the UF line, as highlighted by the effluent NH_4^+ and NO_3^- shares 5.2 mgN L⁻¹ and 4.1 mgN L⁻¹ for ammonia and 4.9 mgN L⁻¹ and 6 mgN L⁻¹ for nitrate (Domínguez-Félez et al., 2024). Following day 22 the microorganisms recovered from the stress condition, achieving higher denitrified N fractions (on average 55 %) and thus reducing the N_2O EF.

In terms of the carbon footprint (Fig. 6), differences between the two lines were more evident. Line II exhibited a slightly higher carbon footprint (4.3 kgCO₂ m⁻³) compared to Line I (3.8 kgCO₂ m⁻³). The increased footprint in the UF line was mainly attributed to higher indirect emissions, which account for 62 % (2.6 kg CO₂ m⁻³), associated with the energy demands of recirculation pumping. In contrast, the MBR line benefited from gravity-assisted recirculation, minimising auxiliary energy requirements and accounting for 50 % (1.9 kg CO₂ m⁻³) of the indirect emissions. These results are aligned with the literature in terms of different emission contributions. However, the UF system registered a higher energy consumption in both lines, and energy consumption represented the major percentage. The energy consumption accounted for 26 % and 32 % of direct emissions in Line I and II, respectively. Moreover, Lines I and II showed similar direct emissions, accounting for 1.18 kg CO₂ m⁻³ and 1.19 kg CO₂ m⁻³, which is higher than those reported in the literature. In both lines, the largest fraction is represented by the CO₂ consumed due to endogenous respiration (CO_{2,Endog}). When comparing our results with those of Bosco Mofatto et al. (2024), although similar values were obtained for the other direct emission fractions (CO_{2,OrgOx} and N₂O), the system showed a lower CO_{2,Endog}. This difference is related to the lower total suspended solids concentration compared to that in our study. Nonetheless, the total carbon footprint values for both configurations remained lower than those typically reported for full-scale conventional WWTPs, reinforcing the environmental sustainability of hybrid IFAS-based systems under optimized operational conditions.

3.3. Microplastics removal

Fig. 7 shows the MPs concentration trends. As shown, influent PE concentrations were maintained at approximately 10 mg L⁻¹ through controlled dosing. Effluent concentrations were remarkably low, measuring 0.018 ± 0.017 mg L⁻¹ in Line I (MBR) and 0.019 ± 0.012 mg

L⁻¹ in Line II (CAS-UF). These concentrations correspond to an overall removal efficiency of 99.8 % in both treatment lines. The cumulative mass balance indicates that of the 168.5 g of polyethylene introduced into the system, over 168.2 g were effectively removed, confirming the robustness of the removal mechanisms in both configurations. These removal rates are consistent with the highest removal efficiencies reported in the literature, despite variations in influent concentrations and analytical methodologies.

For instance, Simon et al. (2018) reported removal efficiencies of approximately 98 % in full-scale WWTPs, with influent MPs ranging from 4.4 µg L⁻¹ to 1.2 µg L⁻¹. Similarly, Xu et al. (2023) documented mass removal efficiencies of MPs exceeding 93 % using pyrolysis-GC/MS analysis techniques. To better understand the membrane removal efficiency, the reactors in which MPs were measured, namely the IFAS reactor and the secondary clarifier, must be analysed. Analysis of intermediate sampling points revealed that a significant portion of MPs was retained during secondary treatment stages. Concentrations measured in the IFAS reactor (0.040 ± 0.032 mg PE L⁻¹) and in the secondary clarifier (0.043 ± 0.047 mg PE L⁻¹) suggest that the biofilm and suspended biomass matrix played a crucial role in the entrapment of MPs. The high standard deviation measured indicates a high variability in MPs concentration over time. In fact, both intermediate points showed peaks of 0.1 mg PE L⁻¹ on day 8, while the lower value was 0.01 mgPE L⁻¹ registered on day 24 and 29. However, the IFAS reactor and secondary clarification further contributed to the removal of MPs before the final filtration steps, achieving a 99.6 % removal efficiency.

Table 3 summarises recent literature findings on the removal of MPs across various WWTP configurations. While methodologies differ, a general trend is confirmed: preliminary and primary treatments remove approximately 50–85 % of MPs, while secondary treatments account for additional removal of 8–35 %, and tertiary treatments remove 2–8 % of remaining MPs (Fauzi et al., 2024; Maw et al., 2024). All studies confirm the high removal efficiency of wastewater treatment plants; however, the challenge of comparison persists due to the absence of a standardized method that would enable a definitive determination of the MP concentration at various stages of the treatment process. Most studies focus on identifying the shapes and colours of MPs. Yet, there is a clear need for a more holistic approach to understanding the mass concentration of these pollutants and the potential effects they may have during the different treatment stages. The high overall removal observed in this study may be attributed to the combined effect of attached and suspended biomass within the IFAS system, enhanced solid-liquid separation in both the clarifier and membranes, and the relatively hydrophobic characteristics of PE, which facilitate biofilm adhesion.

4. Conclusions

This study presented the results of a monitoring campaign conducted

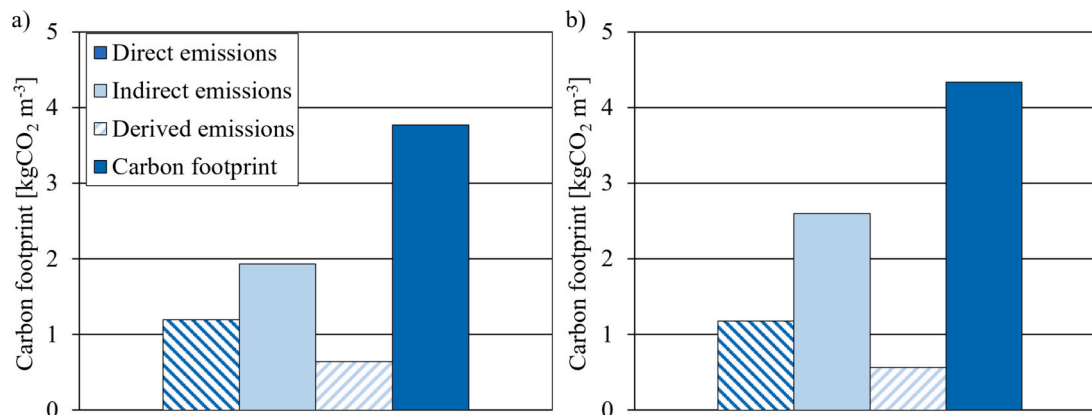


Fig. 6. Carbon footprint and contribution of direct, indirect and derived emissions for MBR (a) and UF (b) lines.

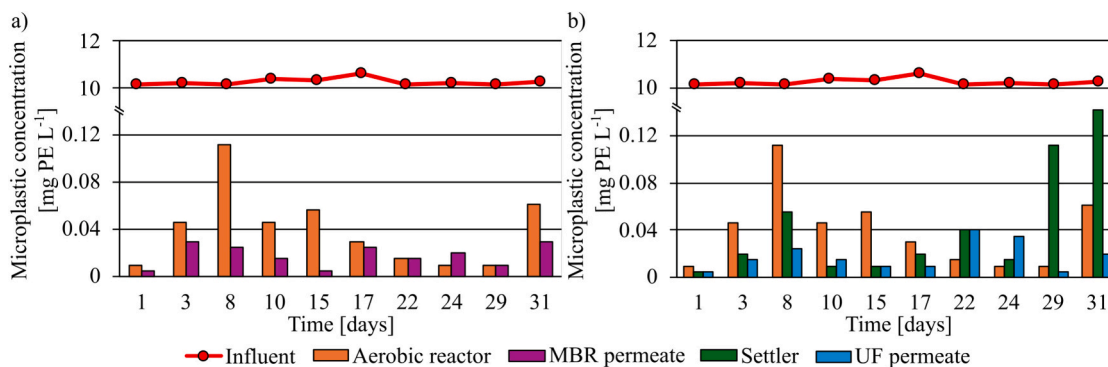


Fig. 7. Microplastic concentration trends in Line I (a) and Line II (b).

Table 3

Average MPs influent and effluent concentrations and removal efficiencies reported in the literature.

MPs type	Configuration	Reactor scale	Influent concentration	Effluent concentration	Removal efficiency [%]	Reference
Mix	CAS	Full	$343.5 \pm 308.79 \mu\text{g L}^{-1}$	$4.4 \pm 4 \mu\text{g L}^{-1}$	98.3	Simon et al. (2018)
Mix	A2O + SF A2O + MBR	Full	$26.23 \pm 7.71 \mu\text{g L}^{-1}$	$1.75 \pm 0.02 \mu\text{g L}^{-1}$	93.3	Xu et al. (2023)
Mix	CAS + filtration	Full	$537.50 \pm 35.21 \text{ particles L}^{-1}$	–	76.7–83.8	Fauzi et al. (2024)
Mix	A2O + UV	Full	$47.06 \pm 37.63\text{--}69.44 \pm 41.00 \text{ MP L}^{-1}$	$4.06 \pm 3.06\text{--}5.44 \pm 3.51 \text{ MP L}^{-1}$	89–93	Zhaxylykova et al. (2024)
PE	IFAS-MBR IFAS-UF	Pilot	10 mg L^{-1}	$0.002 \pm 0.001 \text{ mg L}^{-1}$	99.8	This study

on a pilot-scale IFAS system coupled with two parallel treatment lines: MBR and UF. The results confirmed that both configurations achieved high efficiencies in carbon and nutrient removal. In particular, the UF line demonstrated slightly better total nitrogen removal performance, achieving effluent concentrations compliant with the new limits imposed by Directive 2024/3019. Both lines exhibited excellent microplastic removal capabilities, with overall removal efficiencies of 99.8 %, even under artificially increased influent concentrations of polyethylene particles (10 mg L^{-1}). These results confirm the robustness of IFAS-based systems in mitigating emerging micropollutants.

Nonetheless, the energy demands associated with UF highlight the need for further optimisation to minimise operational costs and environmental impacts. Additionally, while this study demonstrated the high effectiveness of MPs removal under controlled conditions, future investigations should explore the fate of smaller MPs and nanoplastics, which current extraction and analysis protocols may not fully capture. The development of standardized methodologies for MPs quantification remains an essential priority to enable reliable cross-study comparisons. Since wastewater treatment plants are hotspots for MPs, it is crucial to continue researching increasingly efficient technologies capable of eliminating this pollutant and achieving the goals set by the new directive. Overall, this work supports the potential of hybrid IFAS-MBR systems as effective solutions to meet future wastewater treatment challenges, particularly under tightening regulations addressing emerging contaminants.

CRedit authorship contribution statement

Marika Carnesi: Writing – original draft, Investigation, Formal analysis, Data curation. **Antonio Mineo:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Sara Amata:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Antonio Palumbo Piccionello:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Carla Rizzo:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Giorgio Mannina:** Writing – review & editing, Visualization,

Validation, Supervision, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Italian Ministry of University and Research (MUR) through the PRIN 2022 PNRR project, entitled “Innovative Membrane Technologies for Advanced and Sustainable Wastewater Treatment in View of Boosting a Circular Economy Approach” (CUP B53D23027250001). The authors thank ATeN Center at the University of Palermo and Dr. Alberto Spinella for NMR experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biteb.2025.102374>.

Data availability

Data will be made available on request.

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