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De-inked paper sludge and mature compost as high-value components of soilless substrate to support tree growth

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ABSTRACT

The recycled paper industry produces tons of waste whose disposal is a cost for industry and the environment. This research examines the suitability of de-inked paper sludge (DPS), after pelletization, as a sustainable alternative component to a peat-based growing media, creating ideal root conditions for tree development (e.g. high water storage, low compaction). DPS, tested on *Lepidium sativum* L. germination, did not show toxicity effects. Three species, *Quercus ilex* L., *Lagerstroemia indica* L. and *Prunus serrulata* "Kanzan", were planted in 40 cm Ø pots filled with a control (peat, pumice and zeolite) and the experimental substrate (compost, DPS pellets, pumice and zeolite). After two years in the nursery, the trees were planted *in situ*. The physical and chemical properties of substrates were analyzed. Plant morphological and physiological parameters were monitored: trunk diameter, leaf dry matter, leaf nitrogen, chlorophyll, and photosynthetic efficiency. The new substrate showed higher C_{org} (+135%), total N (+73%) and easily available water (+19%), compared to the control substrate used in the nursery. In this new substrate, the trees showed similar radial growth values to the control in the nursery and after transplanting *in situ* improved their photosynthetic performance in terms of quantum yield of photosystem II (+36%, and +29% in *P. serrulata* and *L. indica*, respectively) and electron transport rate (+39%, +25%, and +32% in *P. serrulata*, *Q. ilex* and *L. indica*, respectively). Pelletization represents an attractive amendment for growing media, which enhances the plant's physiological health status. This study proposes alternative recovery methods for paper industry waste with low environmental impact. As the process is developed locally, it should also contribute to reducing energy-related CO_2 emissions from transport. Pelletization represents an attractive novelty in the use of DPS as amendment for growing media, which enhances the plant's physiological health status. This study proposes alternative recovery methods for paper industry waste with low environmental impact. As the process is developed locally, it should also contribute to reducing energy-related CO_2 emissions from transport.

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1. Introduction

Deinked paper sludge (DPS) is derived from recycled paper production, and differs from sludge produced from virgin wood due to the high ash content (Likon and Trebše, 2012). The amount of DPS generated during the production of recycled fibers has been detailed in a few recent studies and official reports. Deviatkin et al. (2016) reported up to 150 kg dry solids/t of paper produced, and Bajpai (2015), described varying amounts of sludge from 50 to 600 kg/t on a dry mass basis, depending on the different paper grades from recycled fiber. The DPS generated worldwide generally ends up in landfills, which not only has a negative effect on the environment (gas and leachate generation), but also involves losses of valuable material contained in the DPS. New treatment technologies and modifications in the processes (digestion, aeration, oxidation, heating treatments, anoxic treatments, filtration and membrane reactors) have gained increasing importance by minimizing sludge generation and reducing the disposal costs (Simão et al., 2018). Sustainable DPS recycling has also been extensively explored for construction, as a component of cement, rock wool (Deviatkin et al., 2016) and bricks (Singh et al., 2018), for energy (Lou et al., 2012) and biogas generation (Amare et al., 2019) and

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environmental technology (Elloumi et al., 2016).

Today the use of peat moss is limited or banned in many countries. This is due to the threat to the fragile habitat biodiversity and the massive release of carbon into the atmosphere when used as a fuel to produce electricity and heat (Carlile et al., 2015; Höök, 2020), and thus its usage also needs to be reduced in the agricultural sector. Several studies have been conducted to find ways to add biological and agronomical value to DPS as well as to find a substitute for peat, linking the production of sustainable growing media with the need to reduce the cost of waste disposal. The high fibrous content of DPS makes it ideal for improving the physical properties (e.g. porosity, water-holding capacity, aeration) of nursery growing media (Méndez et al., 2015), as its high cellulosic content enhances the amount of organic matter (Likon and Trebše, 2012). Other studies were carried out on the employment of DPS in growing media for grass and legumes (Fierro et al., 1997), herbaceous ornamental plants (Chrysargyris et al., 2019), and for the production of lignocellulose (Vodovnik et al., 2018) and fungi (Das et al., 2016). Vannucchi et al. (2018) used DPS as a component of growing media for extensive green roofs, which either mixed with compost or not mixed, reduced the availability of nitrogen, improved the water-holding capacity, and promoted substrate aeration. The porosity of waste materials, such as paper sludge and municipal compost, provides higher gas and water exchanges than other substitutes such as bark (Chong, 2005). A few studies have been carried out with DPS as a substrate amendment for tree cultivation (Filitrault et al., 2006), showing similar or even better properties, than other non-sludge media for trees (Chong and Lumis, 2000). There is limited information on the pelletization of DPS and its role in supporting tree growth. Compost enhances the nutrient content of the growing media (Scharenbroch and Watson, 2014), and improves the soil porosity and structure (Grosbellet et al., 2011). If pumice and zeolite are also added this gives the substrates higher macro and micro-porosity thereby improving the retention of water and cation-exchange capacity (Ramesh and Reddy, 2011) and enhances nitrogen nutrient efficiency (Crippa et al., 2019).

Selecting suitable production practices in the nursery, including the growing media, is important for dealing with post transplanting stress (Close et al., 2005). In fact, transplanting entails changing from the ideal conditions of a nursery to urbanized areas, where tree establishment can be also limited by the quality of urban soil often poor in its structure, containing alien materials, and suffering from compaction and pounding (Bretzel et al., 2020). Urban conditions cause plant stress, which impacts negatively on photosynthetic efficiency and photoinhibition due to excess irradiance, which is then associated with environmental stresses such as low temperature, water-logging or drought (Close, 2005). Suitable substrate properties can help to sustain plant growth after transplanting. The increase in nitrogen content, in particular, improves the photosynthetic activity (Bassi et al., 2018) and the nitrogen storage in plant leaves, thus promoting the biomass production (Liu et al., 2018). The high water-holding capacity in growing media helps to overcome water stress, which would otherwise lead to root elongation (Struve, 2009).

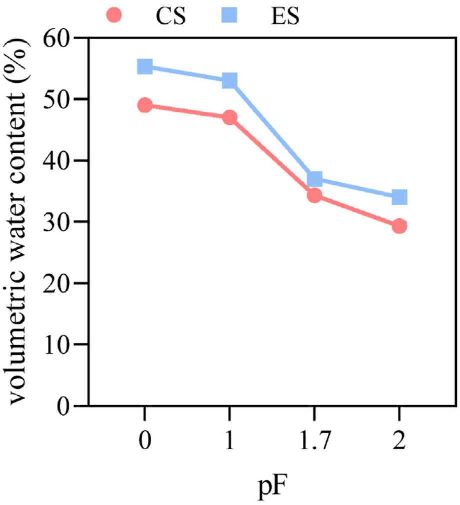

Fig. 1. Water tension curves of the control substrate (CS) and the experimental substrate (ES). The representative points are the moisture content at complete saturation (pF = 1), at field capacity (pF = 1.7) and at wilting point (pF = 2), respectively for the soil water tension (pF) 1 = 10 cm; 1.7 = 50 cm; 2 = 100 cm.

Of the ornamental trees commonly cultivated in urban contexts, *Quercus ilex* L. (Holm oak) is an evergreen species native to the Mediterranean basin. It adapts well to pedoclimatic conditions and is planted in towns and cities where it also mitigates against airborne particulate pollutants all year round (Blanusa et al., 2015). *Lagerstroemia indica* L. (Crape myrtle) is deciduous, originating from tropical Asia and is found as an ornamental species in temperate and sub-tropical climates from the far east to the Mediterranean, and in South America and Australia. Thanks to its small size (2–3 m height) and its colorful summer flowering, it is often planted in urban streets (Chakre, 2006). *Prunus serrulata* "Kanzan" (Kanzan cherry), often obtained by grafting, is a cultivar of *Prunus serrulata* native to China, India and Korea. It can reach 10–12 m in height and displays double flowers in spring, making it suitable as an isolated specimen on open lawns or in avenue plantings and residential gardens.

The DPS used in this study came from the production of recycled tissue paper in Italy and is generated by a mechanical cleaning process, without additives, such as chlorides, and the industrial process produces 48,000 t of waste per year. In Italy, DPS is on the non-dangerous special waste list and can be used for environmental purposes within threshold limits for pollutant concentrations in accordance with Italian legislation (DM 22/1998).

The aim of this research was to support the hypothesis that DPS could be used as an alternative component to a peat-based growing media, used to sustain the post-transplanting of urban trees. To test this hypotheses, the following aspects were assessed: 1) DPS chemical properties and the absence of toxic elements; 2) the substitution of peat in a growth substrate composed of a certified mature compost from green municipal waste, pumice, and zeolite and its effect on the growth of three species of ornamental trees

Table 1
Properties of the materials used as components of the experimental substrate (ES); MGWC = municipal green waste compost; DPS = deinking paper sludge.

Material	pH	C_{org}	N_{tot}	EC	Hg	Cd	Ni	Pb	Cu	Zn	Chlorides
	(H_2O)	(%)	(%)	(mS cm^{-1})	(mg kg^{-1})						
MGWC	7.5	22.3	1.05	1.3	<0.2	<0.5	30	36	42	106	n.d.
DPS	8.0	19.0	0.20	1.9	<0.1	0.2	<10	<0.5	50	38	85.3*

* mg l, migration test in H_2O; n.d. = not detected.

Table 2
Properties of the substrates recorded at the beginning of the experiment in December 2017 and in May 2018. CS = Control substrate; ES = Experimental substrate. EC = Electron Conductivity; CEC=Cation Exchange Capacity; BD=Bulk Density; EAW = Easily Available Water; WS= Water Storage; AW = Available Water. Data are means of 9 replicates ±SD. Asterisks represent significance of $p < 0.05$; ns = not significant; * = $P < 0.05$; *** = $P < 0.001$.

	2017			2018		
	CS	ES	*t*-test	CS	ES	*t*-test
pH (H_2O)	7.4 ± 0.01	7.2 ± 0.02	ns	7.5 ± 0.11	7.5 ± 0.11	ns
EC (mS cm^{-1})	2.9 ± 0.1	6.2 ± 0.02	***	2.5 ± 0.40	2.2 ± 0.54	ns
C_{tot} (%)	8.3 ± 1.6	19.5 ± 1.1	*	6.7 ± 1.47	13.0 ± 2.37	*
N_{tot} (%)	0.63 ± 0.04	1.09 ± 0.02	***	0.31 ± 0.06	0.63 ± 0.12	***
C/N	14.1 ± 3.04	18.5 ± 0.57	ns	21.8 ± 3.38	20.6 ± 1.19	ns
CEC (cmol kg^{-1})	31.4 ± 1.4	30.6 ± 1.4	ns	42.0 ± 3.8	42.0 ± 3.0	ns
BD (g m^{-3})	0.6 ± 0.2	0.5 ± 0.1	*	-	-	-
EAW	13.1 ± 0.65	15.6 ± 0.39	*	-	-	-
WS	4.5 ± 0.07	3.1 ± 0.36	*	-	-	-
AW	17.6 ± 0.63	18.8 ± 0.05	ns	-	-	-

Table 3
Percentage of germinated seeds, mean root length and the germination index (GI) measured in *Lepidium sativum* L. after 72 h in the control (filter paper) and DPS. GI is reported as the ratio of GI estimated in the deinked paper sludge (DPS) samples to the mean GI in the control samples (Hoekstra et al., 2002). Data are means of five replicates ±SD and Asterisks represent significance of $p < 0.05$; ns = not significant; *** = $P < 0.001$.

	Germinated seeds (%)	Mean root length (mm)	GI (%)
control	56 ± 3.20	6.7 ± 1.57	97.4
DPS	44 ± 2.01	13.3 ± 2.02	150.3
t-test	ns	***	

widely used in urban green infrastructure of the Mediterranean; 3) the use of DPS pelletization with improved handleability and substrate properties; 4) the effect of the alternative substrate in supporting tree establishment after transplanting.

2. Materials and methods

2.1. Experimental trial

The experiment was set up in December 2016, at the Marino Favilla nursery (Lucca, Italy, 43°51′05.4″N 10°32′55.3″E). The plants remained for 24 months in outdoor conditions at the nursery and in March 2019 were then planted *in situ*. Two types of substrates were employed: a control substrate (CS) and an experimental substrate (ES). The CS was the substrate usually employed by the nursery, made up of natural blond peat moss 60%; pumice 35%, and zeolite 5%, the acidity of peat was corrected by dolomite lime (in traces). The ES consisted of certified mature municipal green waste compost (MGWC) 62%, pelletized DPS 20%, pumice 13%, and zeolite 5%; all percentages are reported in volume. The addition of 20% DPS is in line with Italian legislation which defines DPS as non-dangerous special waste (waste code 03 03 10), and a maximum of 30% (DM 22/1998) is allowed for use in environmental restoration mixed with matrices such as soil.

A study conducted in Canada (Chong 2005) showed that the very low levels of nitrogen DPS can lead to a deficiency in plants, as microbes tend to consume it. If the amount of DPS is higher than one third of the mix, fertilization is thus needed. The same study reviewed the use of raw or composted DPS, demonstrating that the time and cost of composting is not worthwhile, as it does not lead to a sufficient improvement in the properties needed for growing media.

In our experiment, DPS underwent a process of pelletization in order to make the material and the growing media easier to handle. When the sludge comes out from the production process, it is fluffy, with a very high vol/wt ratio (3.6 ± 0.17) compared to pellets (1.20 ± 0.02). The shape of the pellet also enables water to be retained and creates voids for gas exchanges (Vannucchi et al., 2018). For the compression, the humidity of the waste material should be in a certain range which in this study was fixed at around 20%, thus water was added to reach this percentage.

Eighty-four trees consisting of three species commonly employed in urban planting in a Mediterranean climate were chosen: 28 *Q. ilex* L. (Holm oak), 26 *L. indica* L. (Crape myrtle), and 30 *P. serrulata* “Kanzan” (Kanzan cherry).

Each of the tree species were planted in 40 cm diameter plant pots filled with one of the two substrates, and placed in a completely randomized experimental design ($n = 14, 13, 15$ pots filled with CS, and the same number of pots filled with ES, respectively for *Q. ilex*, *L. indica* and *P. serrulata*). At the beginning of the trial, the *Q. ilex* and *L. indica* were ten years old, and the *P. serrulata* were six years old. Each species had the same trunk diameter: 6.5 ± 0.6 and 6 ± 0.6 cm for *Q. ilex* ($P = 0.62$); 10 ± 0.8 and 10 ± 1.5 cm for *L. indica* ($P = 0.50$); and 9 ± 0.4 and 9 ± 0.6 cm for *P. serrulata* ($P = 0.66$), in CS and ES, respectively. During the two years of the nursery experiment, the critical climate periods occurred in August (max temperatures: 33.2 °C in 2017 and 32.8 °C in 2018; rainfall: 17.2 mm in 2017 and 9.6 mm in 2018). Rescue irrigation was carried out in summer drought conditions (5 L/pot) by a droplet system (May to September), apart from August when it was carried out three times a week. Fertilization was performed and evenly distributed using Nutrient G (dry blood, 12% nitrogen) 0.2% (v/v) twice a year, in early spring and early summer.

In March 2019, after about two years of growth in the nursery, *L. indica* ($n = 26$) and *P. serrulata* ($n = 26$) plants were transplanted to the open spaces of the research area of the National Research Council in Pisa (Italy, lat 43°43′N, long 10°23′E). *Quercus ilex* plants ($n = 10$) were transplanted to the garden of a primary school in Montecarlo (Lucca, Italy lat 43°51′N, long 10°39′ E) among 50-year-old maritime pines, as the plan is to cut down the pines after the *Q. ilex*, which grow well under the canopy of Mediterranean pinewoods, have fully developed.

2.2. Substrate characterization and monitoring

The initial properties of the CS were: pH = 6; electrical conductivity (EC) = 4.0 mS cm^{-1}; bulk density (BD) = 0.28 g m^{-3}; total porosity = 80% (v/v). The physical and chemical properties of the DPS and MGWC used in the ES are reported in Table 1. Pumice and zeolite (Zeolite EPR, commercial name, composed of chabasite and phillipsite) had the following properties: BD = 0.7 (g m^{-3}); water retention = 60%; cation exchange capacity (CEC) = 25–30 cmol kg^{-1} for the pumice and water retention = 30–40%, and CEC = 180–220 cmol kg^{-1} for the zeolite.

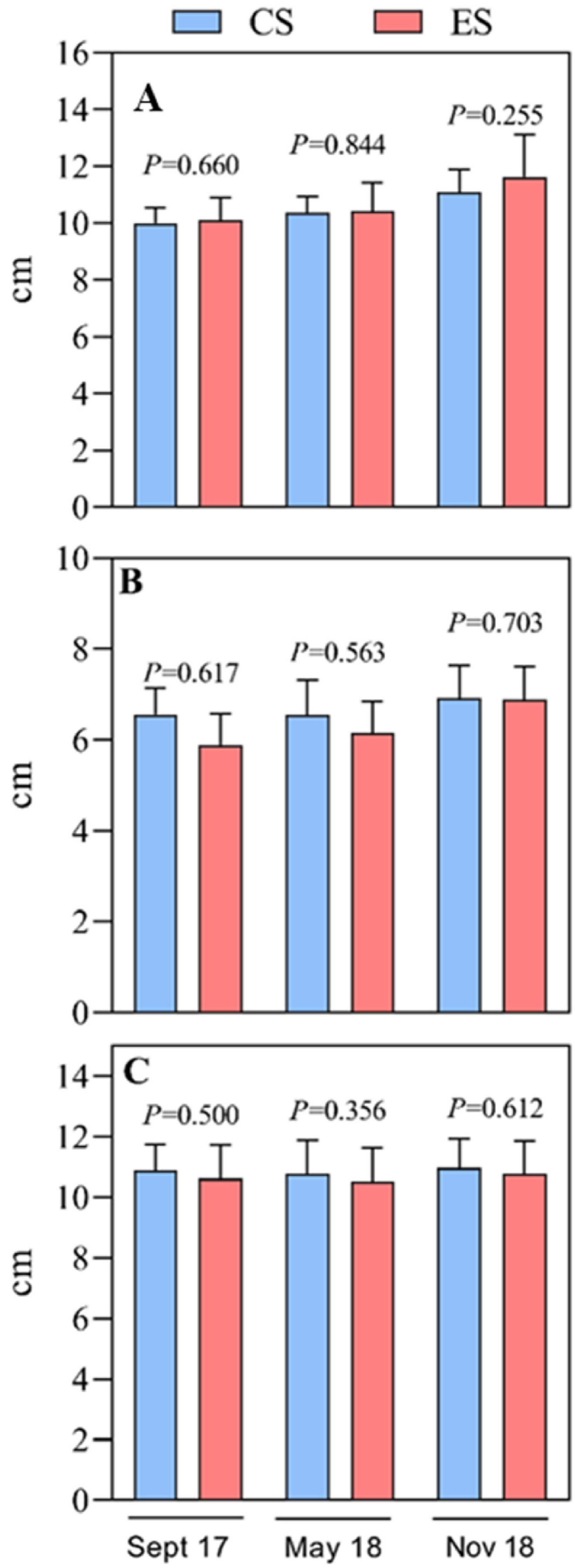

Fig. 2. Trunk diameter measured in *P. serrulata* (A), *Q. ilex* (B) and *L. indica* (C), growing in control substrate (CS) and in experimental substrate (ES). Data are means of 15 (*P. serrulata*), 14 (*Q. ilex*) and 13 (*L. indica*) replicates + SD.

All the values certified by the producers were checked (ASA-SSSA, 1996).

The tension curves of the two growing substrates (CS and ES) were obtained using the UNI-EN 13041 (2011) method, in order to verify their values of easily available water (EAW), water storage (WS), and available water (AW). During the growth in the nursery, the substrates were sampled at the beginning of the experiment (December 2017) and in May 2019 in order to analyze pH, EC, CEC, BD and total carbon (C_{tot}) and total nitrogen (N_{tot}) through dry combustion using a Leco CHN Analyzer (ASA-SSSA, 1996).

2.3. Phytotoxicity test

To prevent any possible toxic effect of DPS on plant development, a germination test was carried out using a model-sensitive plant species, *Lepidium sativum* L. (water cress). Twenty-five seeds of watercress were placed in Petri dishes, on filter paper moistened with ultra-pure water as a control and with 5 g of DPS, wetted with ultra-pure water and covered with a filter paper. Five replicates for each type of medium were prepared. After 72 h at room conditions, the percentage of germinated seeds was established as the ratio of the number of germinated seeds to the total number of seeds. The root length of each watercress seedling was measured and the germination index (GI) was calculated for each replicate as the number of germinated seeds multiplied by the mean root length. The GI value was estimated as the ratio of GI of DPS samples to the mean GI of control samples (Hoekstra et al., 2002). As no phytotoxicity was found in the test on DPS, no similar tests were carried out on the substrate.

2.4. Plant monitoring

During the experiment at the nursery and after the transplanting *in situ*, each species was monitored to evaluate plant growth and health in each substrate. The trunk diameter was measured at 1.3 m from the ground, in September 2017, May and November 2018 at the nursery and after transplanting in June 2019. The total chlorophyll content was estimated with a SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd., Japan) in June and September 2017, and May 2018 at the nursery and in June 2019, after transplanting. The measurement was carried out in fully sun-exposed, completely expanded, IV and V leaves, avoiding the midrib; the leaves were healthy and not damaged and located on selected branches (Muñoz-Huerta et al., 2013). The SPAD units were converted into surface-based specific units of chlorophyll ($\mu g\ cm^{-2}$) using the transformation proposed by Cerovic et al. (2012), as follows: Chl = (99 SPAD)/(144-SPAD).

In order to assess the plant responses to stress factors during growth in the nursery, leaf nitrogen concentration (LNC) and leaf dry matter content (LDMC) were evaluated as indicators of photosynthetic capacity (Kattge et al., 2009; Smart et al., 2017).

After two years, *i.e.* at the end of growth period in the nursery, LNC (% dry-leaf mass) and LDMC ($mg\ g^{-1}$) were measured, according to the plant traits method proposed by Perez-Harguindeguy et al. (2016). For each species, ten young (photosynthetically active) and fully-expanded leaves were harvested from five plants, collected in plastic bags, and stored at 6 °C in darkness. After 12 h, the fresh weight was detected and the leaf samples were then oven dried at 70 °C for 72 h for the LDMC estimation, which was calculated as the ratio of the oven-dry mass to the water-saturated fresh mass. The determination of total N in leaves followed the same method used for the N determination in substrates (Section 2.2), and was calculated as a percentage of the dry-leaf mass. LDMC was estimated as the ratio of the oven-dry mass to the water-saturated fresh mass. LNC was also measured after transplanting.

Three months after transplanting, the photosynthetic performance was assessed through the measurement of chlorophyll *a* fluorescence using a miniaturized pulse-amplitude-modulated fluorometer (Mini-PAM; Heinz Walz GmbH, Effeltrich, Germany), as previously described (Pompeiano et al., 2017). Fluorescence parameters were determined on fully-expanded and exposed leaves of the upper part of the canopy between 10:00 and 12:00. The quantum yield of photosystem II (PSII) in the light adapted state was determined as $\Phi_{PSII} = (Fm'-Fs)/Fm'$ (Genty et al., 1989), where Fm’ is the maximal fluorescence yield with all the PSII reaction centers in the reduced state, obtained by superimposing a saturation flash of light (about 8000 μmol m^{-2} s^{-1}) during exposure to actinic light, and Fs is the fluorescence yield at the actual reduction state of PSII reaction centers in the light-adapted state. The maximum quantum efficiency of PSII was measured on dark-adapted leaves (about 30 min) as F_v/F_m, where F_m is the maximal fluorescence yield in the dark-adapted leaves after application of a saturation flash of light that completely closes all the PSII reaction centers, and F_v is the variable fluorescence, *i.e.* the difference between F_m and the minimum fluorescence yield in the dark-adapted state (F_0). The electron transport rate (ETR, mmol e^- m^{-2} s^{-1}) was calculated as: ETR = Φ_{PSII} x PPFD x 0.5 × 0.87, where the incident photosynthetic photon flux density (PPFD) was obtained using a PAR quantum sensor positioned close to the measurement position, 0.5 is a factor accounting for the partitioning of energy between PSII and PSI, and 0.87 was assumed as the absorbance coefficient (Maxwell and Johnson, 2000). The mean PPFD value during the measurements was about 1600 mmol m^{-2} s^{-1} for both *P. serrulata* and *L. indica*, while for *Q. ilex* it was about 200 mmol m^{-2} s^{-1} due to the shady environment under the pinewood canopy.

2.5. Statistical analysis

Data were subjected to a two-tail *t*-test in order to determine the statistically significant differences of substrate properties and plant parameters. Before statistical analysis, the normality (Shapiro-Wilk normality test) and homoscedasticity (Bartlett test) of the data were confirmed. When normality distribution was not confirmed, the Mann Whitney *U* test was performed. Percentage data were arcsine transformed before analysis. Statistical analyses were performed using R version 1.0.44 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Substrate properties and phytotoxicity test

The properties of the main components of the ES (municipal green waste compost and deinked paper sludge) are shown Table 1. The potential contaminants (heavy metals and chlorides) showed concentrations below the limits for Italian legislation (DM 22/1998), especially Cu (≤150 mg kg^{-1}). The comparison of substrate (CS and ES) properties highlighted important differences, in terms of chemical and physical parameters, that may affect plant growth. Tension curves (Fig. 1) showed higher ($P < 0.01$) saturation of ES compared to CS, as well as higher ($P < 0.01$) field capacity than CS. ES increased by 19% of EAW value ($P = 0.01$), while showing lower WS ($P = 0.02$) than CS (Table 2). The chemical and physical properties of substrates detected in both years (2017 and 2018) at the nursery, and reported in Table 2, show significantly higher values in ES compared to CS, in particular for C_{tot} (+135%, $P < 0.01$), N_{tot} (+73%, $P < 0.01$) in 2017 and C_{tot} (+94%, $P = 0.04$), N_{tot} (+121%, $P < 0.01$) in 2018. EC values of the two substrates differed significantly in 2017 (+114% in ES, with respect to the control) and in 2018 a great variability among the replicates (high SD) observed for both substrates resulted in non-significant differences. In terms of bulk density, ES showed a significantly lower value than CS (−16.7%, $P = 0.03$). The results of the phytotoxicity test aimed at preventing any toxic effect of DPS on plant growth, highlighted no significant differences in the percentage of *L. sativum* seeds germinated in DPS, compared to the control test with ultra-pure water (Table 3). Morphological response was observed, as the root length of seedlings increased significantly in DPS with respect to the control test, after 72 h, as confirmed by the high value of GI (+150.3%).

3.2. Tree responses to substrates

Tree growth and health status were monitored by measuring the trunk diameter, which showed similar values in ES and CS (no significant differences), in both years during the nursery experiment (2017 and 2018) and for all the species tested (Fig. 2). To assess the effects of the two substrates on the photosynthetic pigments of trees in the nursery, the leaf chlorophyll content was estimated and expressed as surface-based specific units (Fig. 3A–C). *Prunus serrulata* and *Q. ilex* in ES substrate showed lower chlorophyll content in September 2017 (Fig. 3A and B), in May 2018 *Q. ilex* showed the same values in CS and ES, with no significant differences between them. For L. *indica* (Fig. 3C), the chlorophyll content significantly increased in trees growing in ES substrate both in September 2017 (+33%) and May 2018 (+25%).

With regard to plant functional traits, LNC measured after two years of the nursery experiment, did not show significant differences for any of the three species in either of the treatments (Table 4). Regarding LDMC, a similar trend was observed, with the exception of *P. serrulata* in ES, which decreased its dry mass content with respect to CS. After transplanting, LNC increased significantly (+7%, $P = 0.03$) in *L. indica* in ES compared to CS, while no differences were found for *Q. ilex* and *P. serrulata*.

The results in terms of N content were in accordance with the growth parameter, *i.e*. the trunk diameter did not show significant differences in any of the plants (Fig. 4A). Fig. 4B shows the chlorophyll content after transplanting in the field for all the species studied. *Prunus serrulata* and *L. indica* significantly increased their chlorophyll content in ES compared to CS (+8% and +10%, respectively), while no significant differences were observed for *Q. ilex*.

Table 5 reports the photosynthetic parameters measured after transplanting. The quantum efficiency of photosystem II in the light (Φ_{PSII}) was significantly enhanced in *P. serrulata* (+36%) and *L. indica* (+28%), growing in ES, coupled with an increased electron transport rate (ETR) in all the treated plants (+39%, +25%, and +32% in *P. serrulata*, *Q. ilex* and *L. indica*, respectively). *Quercus ilex* showed higher values of F_{PSII}, associated with a lower ETR, compared to the other species, due to the relatively low photosynthetic photon flux density (PPFD) under the pinewood canopy. *Lagerstroemia indica* grown in ES also increased (+5%) the maximum quantum efficiency of photosystem II (F_v/F_m), while in the other species this parameter was unaffected by the experimental substrate.

4. Discussion

The results showed that using pelletized DPS as an amendment in a growing media composed of mature compost, pumice and zeolite, enhanced handling, and improved the organic matter content and bulk density, compared to the control substrate. These

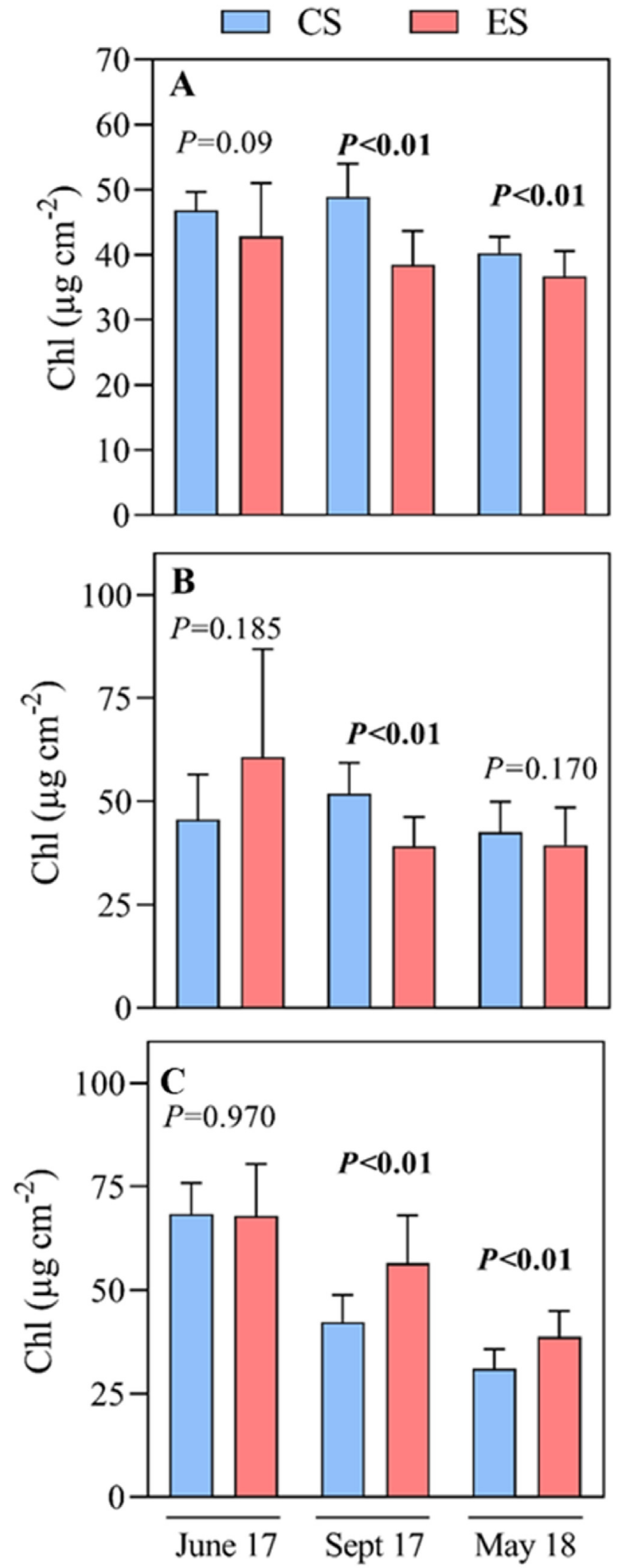

Fig. 3. Chlorophyll content of surface-based specific units (μg cm^{-2}) in leaves of *P. serrulata* (A), *Q. ilex* (B) and *L. indica* (C), growing in the control substrate (CS) and experimental substrate (ES). Data are means of 15 (*P. serrulata*), 14 (*Q. ilex*) and 13 (*L. indica*) replicates + SD. Significant differences are denoted by P values in bold above the bars.

properties, along with a higher water availability and holding capacity, and the good content of nitrogen provided by the compost, enabled healthy nursery plants to grow. The plants grown in the experimental substrate were comparable to the control ones in terms of morphological parameters. On the other hand, after transplanting *in situ* those plants of *P. serrulata* and especially *L. indica* showed an enhanced photosynthetic performance compared to the controls.

In terms of the differences in the chemical properties of the two substrates, the higher values of total carbon in ES were due to the high fiber content in the DPS, which provides a long-term organic amendment (Faubert et al., 2016). DPS provides good properties as an amendment such as CEC, a long-term source of organic matter and water retention, however the very low nitrogen and phosphorus content can lead to a deficiency in plants (Filitrault et al., 2006). Adding compost to the mix counteracted this problem (Lim et al., 2018), as the total nitrogen in ES was higher than the control. The reduction in macro-elements (N and C) in the second year of the experiment, has also been reported in substrates composed of choir, peat or compost. This may be attributed to the absorption and metabolization of these elements by plants and organisms (Fascella, 2015). The presence of compost with an adequate oxygenation of the soil, reduces N_2O emissions into the atmosphere, and reduces the need for fertilization (Lim et al., 2018). The C/N increased the second year, as N decreased at a higher rate compared to C.

Nkongolo et al. (2007) found that composted DPS as an amendment enhanced the biomass of bedding plants, compared to the same growing media with fresh DPS, as the composting process stabilizes N fluxes in the material. Although the loss of N did not reveal any deficiencies in the plants, fertilization should be carried out for long-term maintenance as the microbes tend to consume the elements at the expense of the plants (Filitrault et al., 2006).

The good nutritional conditions of ES, along with the very low compaction, as revealed by the bulk density analysis (Al-Shammary et al., 2018), may enable the roots to spread, possibly reducing transplant stress through modification of the root morphology in sensitive species (Correa et al., 2019). Air-filled porosity and water-holding capacity are important for growing media in terms of root zone storage, however the porosity in the regulation of the exchanges may need to be defined (Nkongolo et al., 2007).

The comparable percentage of seed germination in DPS to the control demonstrates that there were no problems of residual phytotoxicity in the sludge. The increased root development of *L. sativum* in DPS suggests that the excess salts in DPS may have stimulated an acclimation strategy, which reduced exposure to stress (Potters et al., 2007), thanks to the plasticity of the root apparatus (Koevoets et al., 2016).

The use of pelletized DPS in ES enhanced the substrate porosity and the easily available water, as found in similar studies where the size and shape of the paper waste sludge in growing media affected the porosity as well as the air and water movements (Chrysargyris et al., 2019), thus reducing the irrigation requirements (Camberato et al., 2006). A high water availability in the substrate is an important factor in the selection of suitable growing media (Barret et al., 2016) as ornamental plant substrates should never reach the wilting point in order to prevent irreparable damage (de Boodt and Verdonck, 1972). The capacity of the substrate to store water is useful once the tree has been transplanted, as this can help to deal with moderate drought during the acclimatization phase.

Although the EC value detected in ES, in the first year was higher

Table 4
Leaf nitrogen concentration (LNC, %) and leaf dry matter content (LDMC, mg g^{-1}) measured in *P. serrulata*, *Q. ilex* and *L. indica*, growing in the control substrate (CS) and experimental substrate (ES) in the nursery. Data are means of five replicates ± SD and asterisks represent significance of $P < 0.05$; * = $P < 0.05$; ns = not significant.

	P. serrulata			Q. ilex			L. indica		
	CS	ES	*t*-test	CS	ES	*t*-test	CS	ES	*t*-test
LNC	2.2 ± 0.15	2.3 ± 0.36	ns	1.2 ± 0.15	1.2 ± 0.11	ns	1.6 ± 0.26	1.3 ± 0.17	ns
LDMC	0.40 ± 0.01	0.36 ± 0.02	*	0.55 ± 0.01	0.52 ± 0.03	ns	0.37 ± 0.01	0.37 ± 0.01	ns

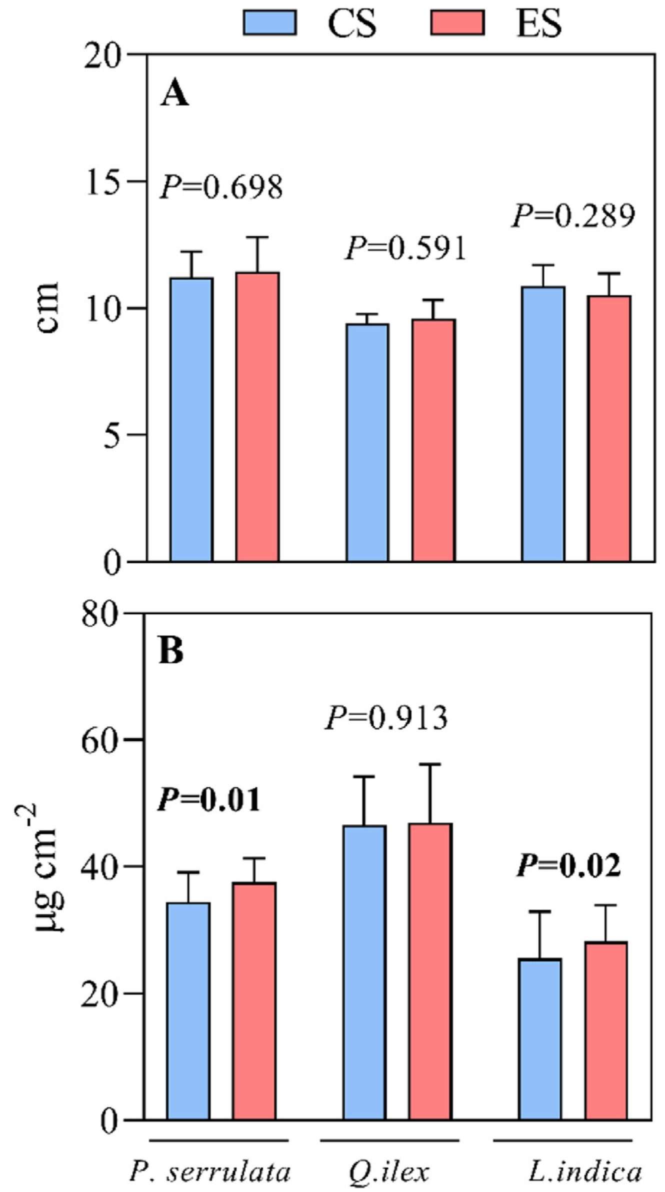

Fig. 4. Trunk diameter (A) and chlorophyll content of surface-based specific units (B), measured after transplanting (March 2019), in *P. serrulata*, *Q. ilex* and *L. indica*, growing in the control substrate (CS) and experimental substrate (ES). Data are means of 10 and 27 (*P. serrulata*), 5 and 15 (*Q. ilex*), 13 and 39 (*L. indica*) replicates + SD for trunk and chlorophyll data, respectively. Significant differences are denoted by P values in bold above the bars.

than the one considered optimal for growing media (0.5 dS/m) (Abad et al., 2001), in the second year the substrates composed of DPS showed a comparable EC to the control substrate commonly used in tree nurseries, and no detrimental effects on plant health were observed. In fact, the EC values in the ES were below 4.0 mS cm^{-1}, indicating that the salt content was not harmful (Chhabra, 2004). EC and potentially toxic element accumulation are the main limiting factors of substrates composed of waste (Carlile et al., 2015), which is why it is key to find the right synergies of different raw materials and to calibrate the percentages in order to obtain a suitable growing media (Fascella, 2015). However, high values of EC, related to the excess salts in DPS, drop quickly after irrigation (Chong, 2005). No high values of toxic metals, overlaying the law limits for this kind of waste, were found in the sludge (DM 22/1998). Tandy et al. (2008) found a high Cu content in DPS employed as an amendment in agriculture, while the raw material, transformed into a substrate mixed with compost, pumice and zeolites, still presented a low content of Cu (about 30 mg kg^{-1}), in line with non-contaminated soils, and in an adequate amount as an essential element for plants. Most nutrients are available at a limited pH, of around 5–6, which is one reason why peat is so commonly used in horticulture (Barrett et al., 2016). Although the pH was around 7 in both CS and ES substrates used in this study, the trees did not show any symptoms of deficiencies.

A high amount of DPS >30% added to substrates can occasionally negatively affect the shoot biomass, plant height and stem diameter, unless fertilizers are used, as seen in *Populus deltoides* and *Alnus crispa* (Filiatrault et al., 2006). The growing media composed by 20% of DPS did not negatively influence the growth of plants in terms of trunk diameter, and the two treatments had the same value in all three species, both in the nursery and after transplanting, in line with Tripepi et al. (1996). During the first vegetative season in the nursery, the LDMC was lower in *P. serrulata*. In fact, in some cases nitrogen availability can affect the values of leaf traits and increase the specific leaf area with nitrogen supply, while it decreases LDMC (Al Haj Khaled et al., 2005).

The chlorophyll content is an important predictor of plant photosynthetic rate (Croft et al., 2017), reflecting the presence of plant stress. The percentage of paper waste sludge can affect the leaf chlorophyll content, as found by Chrysargyris et al. (2019). *Quercus ilex* and *L. indica* showed a good health status in the nursery in terms of chlorophyll indices (Cinelli et al., 2004). In fact, no statistical differences were found for *Q. ilex*, while *L. indica* actually increased the chlorophyll content in ES. The increase in chlorophyll content in *L. indica* probably corresponded to the higher presence of nitrogen in ES, as nitrogen supply has important role in relation to enhancing the chlorophyll content (Bassi et al., 2018), and *L. indica* is an N-sensitive species and responds well to the right amount of N (Cabrera and Deveraux, 1999). The chlorophyll content decreased in *P. serrulata* in ES during growth in the nursery, suggesting a slowdown of chlorophyll synthesis. After transplanting, the main effects of the growing media were in terms of photosynthetic performance and chlorophyll content, which were determined through chlorophyll fluorescence analysis and SPAD units, respectively. In fact, these non-invasive analyses are among the most powerful and widely used techniques to evaluate plant health and plant response to abiotic stress under field conditions (Maxwell and Johnson, 2000; Cerovic et al., 2012).

After the plants had been transplanted, Φ_{PSII}, ETR and chlorophyll content were greater in *P. serrulata* and *L. indica* grown in ES.

Table 5
Photosynthetic parameters measured after transplanting in *P. serrulata*, *Q. ilex* and *L. indica*, growing in the control substrate (CS) and experimental substrate (ES). F_v/F_m = maximum quantum efficiency of photosystem II in the dark-adapted state; Φ_{PSII} = effective quantum efficiency of photosystem II in the light; ETR = Electron Transport Rate (μmol m^{-2} s^{-1}). Data are means of nine (*P. serrulata*), five (*Q.ilex*) and seven (*L. indica*) replicates ± SD. Asterisks represent significance of $P < 0.05$; ns = not significant. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

	P. serrulata			Q. ilex			L. indica		
	CS	ES	*t*-test	CS	ES	*t*-test	CS	ES	*t*-test
Φ_{PSII}	0.11 ± 0.005	0.15 ± 0.01	*	0.51 ± 0.051	0.59 ± 0.020	ns	0.14 ± 0.01	0.18 ± 0.01	***
ETR	71.4 ± 3.25	99.4 ± 8.05	**	34.0 ± 2.84	42.5 ± 2.33	*	94.9 ± 3.79	125.6 ± 8.54	***
F_v/F_m	0.77 ± 0.02	0.78 ± 0.02	ns	0.81 ± 0.009	0.83 ± 0.004	ns	0.77 ± 0.02	0.81 ± 0.01	*

Again, the higher nitrogen content in ES may increase the photosynthetic performance and the chlorophyll content (Bassi et al., 2018) in these species. During the leaf growth the increased nitrogen storage in *L. indica* leaves may help sustain leaf expansion, as well as synthesize photosynthetic proteins (Liu et al., 2018). An enhanced photosynthetic performance reduces the risks of photoinhibition related to transplanting stress, *e.g.* as a consequence of the high irradiance associated with other environmental stress such as low temperature, waterlogging, or drought (Close, 2005) as well as reduced nutrient uptake (Struve et al., 2009). The absence of photoinhibitory processes was confirmed by the optimal values of F_v/F_m close to 0.8 observed in all three species growing in ES (Maxwell and Johnson, 2000), with *L. indica* even showing an enhancement of this photochemical index. In the case of *Q. ilex*, the shade provided by the pinewood canopy increased the photochemical efficiency of photosystem II and reduced the electron transport rate compared to other species, counteracting any possible difference in the effects of the two treatments. Even at a relatively low PPFD, *Q. ilex* grown in ES showed a higher ETR than those grown in CS, suggesting that this substrate has a positive effect on the photochemical reactions in light-limited environments.

Although the effect on stem growth was not observed and the root system was not monitored, after transplanting the trees grown in ES showed a higher photochemical efficiency and/or electron transport rate than those grown in CS, indicating a better physiological health status in ES than in the control. It is generally assumed that transplanted trees experience a phase after planting in which growth is significantly reduced due to a root-shoot imbalance and several other stress factors, such as summer drought, which can affect their survival and recovery rates from transplant shock (Watson, 2005). Transplanted trees should be able to re-establish sufficient roots to sustain themselves and a comparable root/shoot ratio to non-transplanted trees and to counteract possible additional stress, at the expense of radial growth. In any case, the resulting influence on plant growth will be assessed through future monitoring in the years following transplanting.

5. Conclusions

The substrate composed of DPS pellets and mature green waste compost is suitable for sustaining the growth of trees in pots in nurseries and to foster their capacity to counteract transplant stress. In terms of physico-chemical properties, the experimental growing media initially had +73% total N, +135% C, 114% EC, and −17% BD and +19% EAW, compared to the commercial substrate. Some of these advantages were maintained in the following year: ES showed +94% C_{tot} and +121% N_{tot} compared to the control. The experimental growing media maintained good plant physiological health status as highlighted by the improved photosynthetic performance, with greater Φ_{PSII} and ETR values than 20%. The C/N value (up to 20) highlights that N fertilization is required over time. The pelletization of the DPS was successful in improving the handling of the sludge and the substrate. The DPS used in this study is produced near Lucca which is very close to a large nursery district (Pistoia) where there are more than 5200 ha and 1500 nurseries of ornamental plants, consequently, the waste could be recycled with a low carbon-emission and environmental impact from transport. This study represents a valid and replicable example of the circular economy, not only at a local level, but also at a global level in geographical areas where waste from the paper industry can be exploited in nursery production. Future research will focus on investigating the role of pelletized DPS in improving other substrate properties, such as stability, increasing the amount of DPS and employing different components, such as choir, to obtain a commercial formula.

CRediT authorship contribution statement

Francesca Vannucchi: Formal analysis, Investigation, Resources. **Andrea Scartazza:** Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Manuele Scatena:** Formal analysis, Investigation, Resources, Data curation. **Irene Rosellini:** Formal analysis, Investigation, Resources, Data curation. **Eliana Tassi:** Writing - original draft, Writing - review & editing, Visualization. **Fabrizio Cinelli:** Conceptualization, Methodology, Validation. **Francesca Bretzel:** Writing - original draft, Writing - review & editing, Visualization, Supervision.

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.

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