Ecological Indicators 150 (2023) 110267

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Do electromagnetic fields used in telecommunications affect wild plant species? A control impact study conducted in the field

Marek Czerwiński [a,*], Alain Vian [b], Ben A. Woodcock [c], Piotr Goliński [a], Laura Recuero Virto [d], Łukasz Januszkiewicz [e]

[a] Department of Grassland and Natural Landscape Sciences, Poznań University of Life Sciences, ul. Dojazd 11, 60-632 Poznań, Poland
[b] Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France
[c] NERC Centre for Ecology and Hydrology, Crowmarsh Gifford, Wallingford, Oxfordshire OX13 6NT, UK
[d] Léonard de Vinci Pôle Universitaire, Research Center, 92916, Paris La Défense & i3-CRG, École polytechnique, CNRS, IP Paris, France
[e] Institute of Electronics, Lodz University of Technology, ul. Wólczańska 211/215, 90-924 Łódź, Poland

ARTICLE INFO

Keywords:
Microwave radiation
Wireless communication
Base station
Ecological effects
Clover
Trifolium

ABSTRACT

Over the last three decades there has been an unprecedented increase in both the coverage of wireless communication networks and the resultant radiofrequency electromagnetic field (RF-EMF) exposure level. There is growing concern that this rapid environmental change may have unexpected consequences for living organisms. Existing research on plants has shown that RF-EMF radiation can affect their growth and development, gene expression and various metabolic activities. However, these findings are largely derived from short-time exposure of crop plants under laboratory conditions. It remains unclear to what extent plants are affected by artificial RF-EMFs in real ecosystems and what potential consequences this could have for ecosystems. This study attempts to assess these long-term effects of RF-EMF exposure on wild plants under controlled experimental field conditions. We investigated the impacts of RF-EMF exposure (866–868 MHz frequency band) from seed germination to maturation for ten common herbaceous plant species over a four-month period. The selected plant species belong to various families and have different functional and morphological traits that might affect a response to the applied RF-EMF.

For most of the considered species responses to RF-EMF were undetectable or weak, and where present restricted to a single trait. Only for one species, *Trifolium arvense*, were effects observed at different plant development stages and for different plant characteristics. In this species RF-EMF stimulated growth and probably influenced leaf heliotropic movements, as indicated by a larger height, larger leaf area and altered leaf orientation one month after germination. However, over the growing season *Trifolium arvense* plants exposed to RF-EMF entered the phase of senescence earlier, which was manifested through a reduction of green leaf area and an increase in the area of discolored leaf.

We conclude that the effects of RF-EMF exposure at environmentally relevant levels can be permanent and irreversible in plants growing in the open natural environment, however, these effects are restricted to specific species. This in turn suggests that future studies should examine whether the effects observed here occur also in more common *Trifolium* species or other legumes that are a keystone component within European grasslands. Our findings also show that *Trifolium arvense* could be a candidate indicator of man-made RF-EMFs in the environment.

1. Introduction

An unprecedented increase in both the coverage of wireless communication networks and the resultant exposure level of radiofrequency electromagnetic fields (RF-EMF) have taken place over the last three decades. For example, Bandara and Carpenter (2018) reported that the levels of exposure to RF-EMF at the ~1 GHz frequency band, commonly used for wireless communications, have increased by a magnitude of 10^{18} from natural levels. There is growing concern that this rapid environmental change may have unexpected consequences for

* Corresponding author.
E-mail address: m.czerwinski@up.poznan.pl (M. Czerwiński).

https://doi.org/10.1016/j.ecolind.2023.110267
Received 21 February 2023; Received in revised form 13 April 2023; Accepted 14 April 2023
Available online 19 April 2023

living organisms (Levitt et al., 2022; Malkemper et al., 2018; Sutherland et al., 2018; Thielens, 2021). Numerous studies on plants have shown that wireless communication microwave radiation can affect their growth and development, gene expression and various metabolic activities. These effects occur at exposure levels equivalent or less than those often recorded under environmental conditions. Moreover, they were recorded for RF-EMFs differing in wavelength and polarization, continuous or modulated waves, and for different signal modulation types (Cucurachi et al., 2013; Czerwiński et al., 2020; Halgamuge, 2017; Vian et al., 2016). However, the predominance of controlled laboratory-based assessments underpinning these findings means that it remains unclear as to what extent plants growing in real ecosystems are affected by RF-EMFs used for wireless communication. As such, the potential wider consequences of RF-EMFs on whole ecosystems remain unknown. This issue needs to be addressed in field scale studies using plants grown under open conditions and exposed to a range of natural environmental stresses. Further, the current evidence base focuses on crop plants, such as *Vigna radiata* or *Zea mays*, and so neglects wild taxa that underpin complex ecosystems. Another failing of the current evidence base is that most studies relate to plant responses observed for short exposure periods, often in the phase of seed germination or seedling emergence and so neglect longer term assessments over the plant lifecycle (Cucurachi et al., 2013; Czerwiński et al., 2020; Halgamuge, 2017; Kaur et al., 2021; Vian et al., 2016).

In the context of these knowledge gaps, we undertook a study to assess whether environmentally relevant RF-EMF from wireless communication would have long-term effects on common herbaceous plants under field conditions. Using a controlled experimental design, we investigated the impacts of RF-EMF exposure over four-month period, from seed germination to maturation, for 10 herbaceous grasses, forbs and legumes. These plants were exposed to RF-EMF at power flux density ranging from 10 to 20 mW m^{-2}, which was above the expected threshold of responses previously identified (Czerwiński et al., 2020) but a few hundred times lower than precautionary regulatory exposure limits adopted in most countries worldwide for RF-EMF from cellular base stations (World Health Organization, 2022). The irradiation scenario we apply simulated real-life exposure conditions, which are likely to elicit biological response in wild herbaceous plants commonly occurring in suburban and rural areas (Appendix A, Czerwiński et al. 2020). This scenario resembled conditions in places of peak radiation around cellular base stations (on or near the axis of the main radiation beam from a cellular antenna): electromagnetic waves were vertically polarized and fell from one dominant direction, at an acute angle to the ground. We used RF-EMF at the frequency of 866–868 MHz, which was near the low frequency bands commonly utilized in cellular networks: 900 MHz band used in GSM and 800 MHz band used in LTE. We tested the hypotheses:

(1) RF-EMF effects in plants can be observed under field conditions where plants are exposed to natural occurring environmental stressors.
(2) There is inter-specific variation in the extend of RF-EMF effects.
(3) Chronic RF-EMF exposure can produce permanent, irreversible changes in plants morphology.

To verify the last hypothesis we focused on plant height, shape, and leaf area, because these traits have been shown to respond to RF-EMFs under lab conditions (Grémiaux et al., 2016; Halgamuge, 2017; Kaur et al., 2021; Senavirathna and Asaeda, 2014). Additionally, we tested RF-EMF effects on leaf orientation. While there is no evidence of such effects, many species (including *Medicago falcata* and *Trifolium arvense* used in this study) adjust leaf orientation relative to the sun with these heliotropic movements modified by other environmental factors, such as air temperature (Fu and Ehleringer, 1989), water availability, or nitrogen supply (Kao and Forseth, 1992).

2. Methods

2.1. Location of the experiment

The experiment was carried out at a plant nursery located 80 km east from Wrocław, Poland (Biadaszki village, N 51° 15′ 6.8″, E 18° 9′ 50.0″). This site is remote and located in a valley isolated from man-made RF-EMFs by a large forest. The main source of artificial radiation are cellular base stations which are located over four kilometers away. The measurements conducted before and during the study showed an exceptionally low background level of RF-EMF exposure: maximum RMS values never exceed 3 μW m^{-2} within a bandwidth from 700 MHz to 6 GHz typical to wireless communication services including mobile telephony (GSM, CDMA, UMTS, DECT and LTE), Bluetooth, WLAN and WiMAX. Details of the measurements are provided in Appendix A.

2.2. Experimental setup

The experiment was set up on leveled area with no obstructions that could alter the designed distribution of EMF. The area was divided into two equal parts, hereafter referred to as "radiation" and "control", with one experimental plot located within each part. We randomly chose which of these two halves would be allocated to the RF-EMF treatment. Plants grown within the radiation plot were exposed to artificial RF-EMF emitted from a directional antenna mounted on a pole located in the middle of the experiment area, between the radiation and control plot (Fig. 1). The RF-EMF produced from the antenna formed a beam, a so-called "main lobe", which encompassed the radiation plot. There were also some minor backward- and sideward-directed "side lobes" which represented undesired radiation. We used metal screen made from a conductive wire mesh stretched out on a steel frame (1.64 m high and 2.0 m wide) to limit the side lobe radiation effect for the control plot. As this screen produced a slight shadow in the late afternoon, a dummy copy of the metal screen (made of PVC pipes and polyethylene net) was established to replicate this effect for the radiation plot (Fig. 1).

The experimental plots with pots of the studied plants (see below) were located at a distance of at least 3.0 m from the antenna to avoid near-field EMF effects. The division line between the two parts of the experimental area was marked out roughly along a north to south axis, with the two parts located westward and eastward (Fig. 1). Radiation axis was therefore almost parallel to the W-E direction so that the potential RF-EMF effects were unbiased by horizontal leaf orientation resulting from plant responses to the direction of solar radiation. Each pot had one side marked with a color paint. Throughout the period of plant growth, the marked side was directed to the east, so that plants position relative to the antenna remained constant.

2.3. Exposure system

The RF-EMF applied in the experiment was produced by an antenna connected to a commercial RFID reader as a transmitter. This was an UR4 device (Chainway Ltd., China) designed to identify and track passive tags in shops, warehouses, production lines etc. This system used digital transmission, sending data in cyclic frames (slots) that lasted 205 ms separated by 3.3 ms interframe gaps (when no signal was transmitted). The transmitter operated in automatic permanent interrogation mode, switching transmission between four channel frequencies: f_1 = 865.71 MHz, f_2 = 866.31 MHz, f_3 = 866,91 MHz and f_4 = 867,5 MHz (Fig. 2). The signal was modulated in each of these four channels using a phase-reversal amplitude shift keying (GS1 EPCglobal Inc., 2015, p. 27). Detailed characteristics of the applied EMF are given in Appendix A.

Measurements of the EMF characteristics were carried out using a FSH8 spectrum analyzer connected to a TSEMF-B2 isotropic measuring probe (Rohde & Schwarz GmbH & Co., Germany). RF-EMF exposure level was measured using the Max Hold mode. This detected the maximum RMS values occurring every 5 s interval. The recorded values

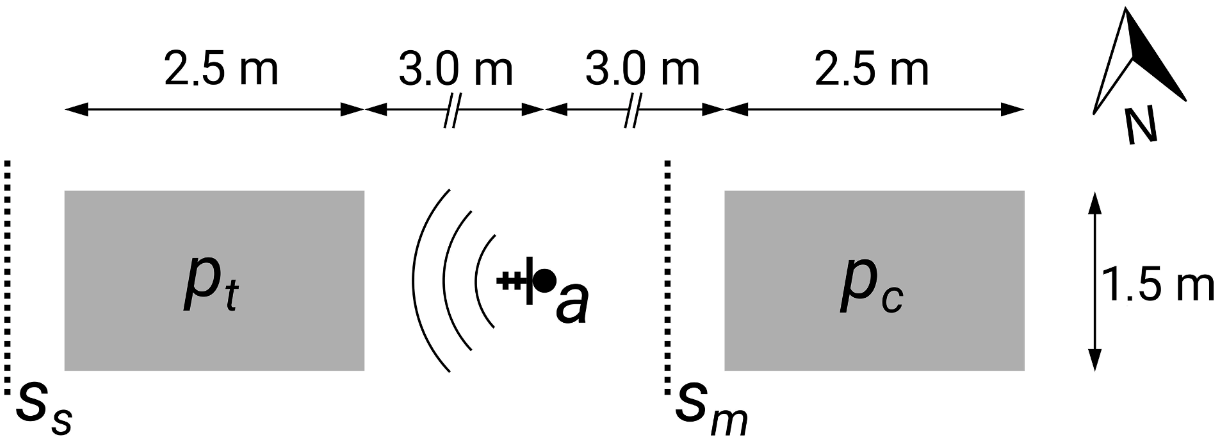

Fig. 1. The experiment setup; symbols used: a – antenna, p_c – control plot, p_t – treatment plot (or "radiation plot"), s_m – electromagnetic shielding screen, s_s – sham screen.

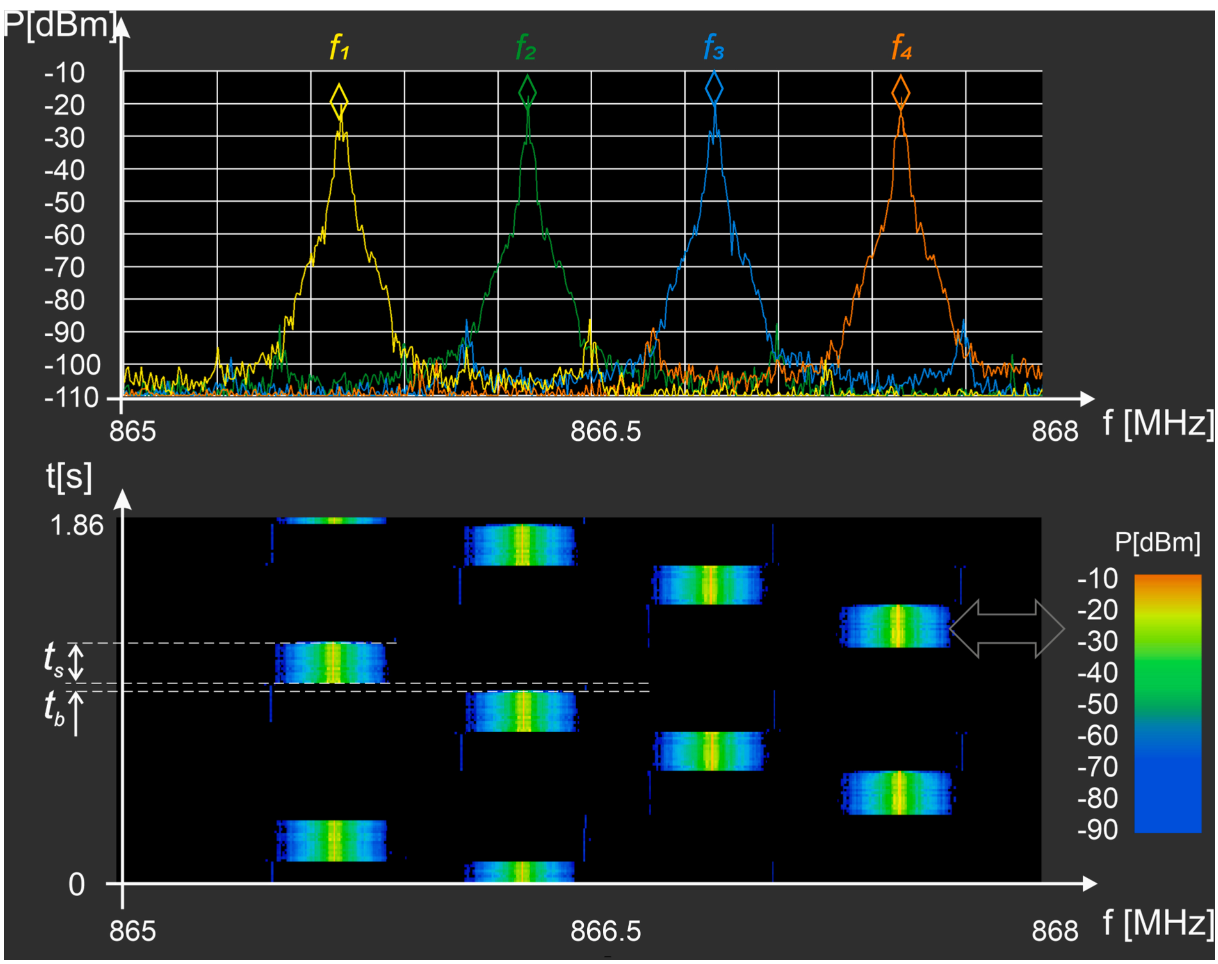

Fig. 2. The spectrogram of the transmitted signal; P – transmission power; t_s – time slot (frame) lasting 205 ms; t_b – 3.3 ms interframe gap; f_1-f_4 designate channel frequencies.

were averaged across the four corners of a plot. Mean power flux density in the radiation plot was 12.4 mW m^{-2} and 16.7 mW m^{-2} at 20 and 40 cm respectively above the ground surface. The same measurements performed within the control plots were 0.003 mW m^{-2} and 0.005 mW m^{-2}. Detailed information on the distribution of the EMF at the experiment site is provided in the Appendix A.

It is worth noting that the RFID exposure system used in our experiment shared technological properties of LTE, GSM and other common cellular communication systems that most contribute to environmental EMF field exposures. It used vertical wave polarization, digital modulation, frequency channel switching and transmission with division into time frames. Also, the frequency band, power density level and emission variation pattern (signal waveform) were very similar to those used in cellular systems. According to the French Agency for Environmental and Occupational Health and Safety (AFSSET), the scientific data, interpretations and conclusions of analyses related to the effects of RF-

EMF on human health based on mobile telephony can be extrapolated to RFID technologies (AFSSET, 2009).

2.4. Species selection

We selected annual plant species that germinate in spring or early summer so that our observations would be completed in one growing season over the entire plant life cycle, from seed to a mature plant. We chose species belonging to various families thus establishing a wealth of functional and morphological traits that may affect a response to the applied EMF (Czerwiński et al., 2020). The selected species differed in terms of plant height, stem architecture (branched vs. unbranched), specific leaf area (SLA), leaf orientation and shape, leaf heliotropism, leaf anatomy (sclero-, meso- or hygromorphic) and seed type (albuminous, perispermic and exalbuminous). These species were: *Avena fatua* and *Setaria viridis* (*Poaceae*), *Berteroa incana* and *Thlaspi arvense* (*Brassicaceae*), *Medicago falcata* and *Trifolium arvense* (*Fabaceae*), *Myosotis arvensis* (*Boraginaceae*), *Polygonum persicaria* (*Polygonaceae*), *Spergula arvensis* (*Caryophyllaceae*) and *Viola arvensis* (*Violaceae*). The selected species are characterized in detail in the Appendix A.

2.5. Cultivation of the studied plants and the estimation of seedling emergence rate and aboveground biomass

Wild occurring seeds of the selected plant species were collected in June and July 2020 from the vicinity of the experimental site where the exposure level to artificial RF-EMFs was very low. After the collection, the seeds were stored under warm and dry conditions until sowing. For four species (*Setaria viridis, Polygonum persicaria, Viola arvensis* and *Trifolium arvense*) appropriate dormancy breaking treatments (damp cold, scarification or imbibition in the presence of gibberellic acid) were applied before sowing. Seeds were sown on May 6, 2021 into a standard organic potting soil (peat), 13 seeds per pot. They were watered once or twice a day, depending on the weather. Plants on both halves of the experimental area were watered with identical amount of water. On each half of the experiment area, the pots were arranged in an array where species were distributed evenly so that average light availability, RF-EMF exposure level, wind speed and other growth conditions were the same for each species (Appendix A).

For the first two months from sowing, plants were grown in 0.4 L pots that made up 15-pot trays. On each half of the experiment area, there were four trays per species. The number of seedlings was counted after one month to assess seedling emergence rate (r_e). Following this the number of plants in each pot was reduced to one individual by uprooting randomly selected seedlings. After this initial reduction, 600 plants (four 15-pot trays per species) remained on each half of the experiment area. The number of plants was reduced again in July to avoid mutual shading. First, we excluded seven species which had either completed their life cycle or started to overgrow the pots. This left the species *Trifolium arvense*, *Viola arvensis* and *Avena fatua*. Of these remaining three, we further reduced the number of plants per species to 24, so that 72 plants remained in each half of the experimental area. The removed plants were cut, air-dried and weighted to determine their aboveground biomass (b). The remaining plants were replanted in 3 L pots (hereafter referred to as “single pots”) and were grown in the experiment area until mid-September.

2.6. Image analysis

2.6.1. Photographing the plants

The plants were photographed three times during their growth: two months after sowing, when the plants grew in multipot trays, and three and four months after sowing, when they grew in single pots. Plants growing in the multipot trays were photographed from four different sides, using different perspective planes: horizontal (east and west side), oblique (approximately 30° east, west, north and south side) and vertical (Fig. 3). Plants replanted to single pots were photographed using only the vertical perspective plane.

The photos were taken against white or brown backdrop, with a ruler located next to a photographed plant to provide a benchmark for further assessments. Images with a resolution of 16 megapixels were taken using Olympus TG-3 camera installed on a tripod.

2.6.2. Plant height and shape determination

Plant height and shape were measured using photos taken from east and west horizontal perspectives. This was done two months after sowing, when plants grew in the multipot trays. The calculated values were averaged to obtain one value per tray. Image mode was converted from RGB to black and white, where black pixels represented foliage and white pixels represented the background. Plant height h (cm) was defined as the height from the baseline of the plants (0 cm) to the level below which 90% of black pixels were located (Fig. 4). Plant shape was determined by the vertical distribution of black pixels in the layer between 0 cm and h level (hereafter referred to as plant canopy profile). This distribution was described using the plant shape index (s), defined as: $s = a/b$, where a and b are the number of black pixels in the upper or

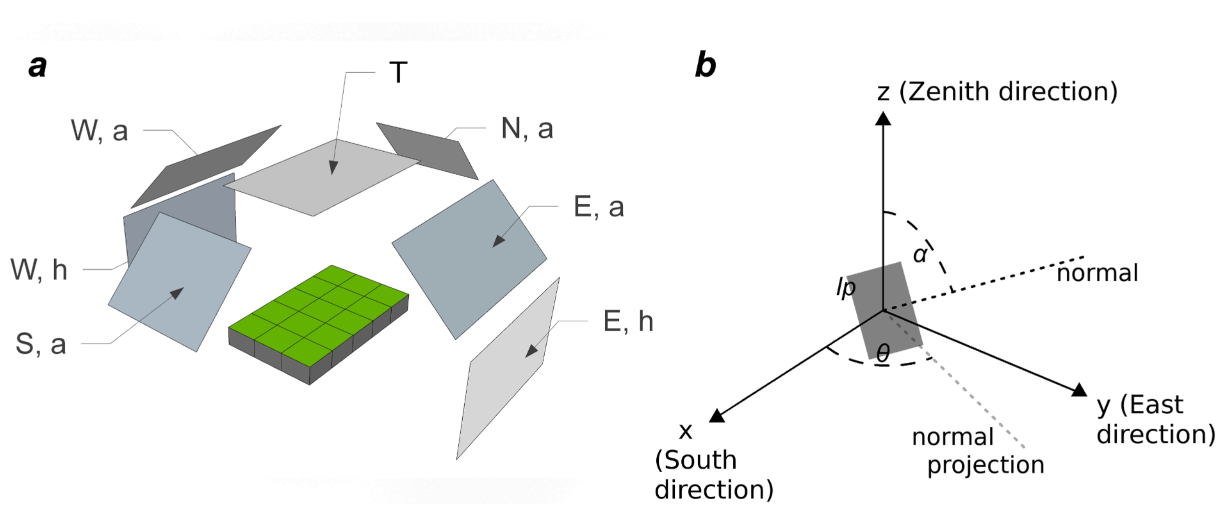

Fig. 3. A. perspective planes used for photographing the studied plants which grew in multipot trays; capital letters denote different sides from which the photos were taken: N – north, S – south, W – west, E – east, T – top; small letters designate horizontal (h) or angled (a) perspective; b. Definition of leaf orientation: lp – leaf plate, Θ – leaf azimuth and α – leaf inclination.

Fig. 4. Plant height and shape determination; height (h) was defined as a vertical distance from the baseline of the plants to the level below which 90% of black pixels are located; a, b – the number of black pixels in the upper or lower half of the canopy profile, respectively.

lower half of the canopy profile, respectively (Fig. 4).

2.6.3. Leaf area measurements

Leaf area was assessed for photographs taken from the vertical perspective at three different times: two months after sowing, when the plants grew in the multipot trays, and three and four months after sowing, when the plants grew in single pots. Leaf area was analyzed with WinDIAS 3.3 software (Delta-T Devices Ltd, UK). It was determined by counting the number of pixels that represented foliage and converting the obtained projection area to surface area expressed in cm^2. Total leaf area (la_t) was comprised of green leaf area (la_g) and discolored leaf area (la_d). The latter was represented by yellowish, reddish or brownish pixels, depending on the species and plant growth stage.

The images taken two months after sowing were used to assess RF-EMF impact on all 10 plant species (see section 3.2). These images were then combined with the images taken three and four months after sowing to investigate the temporal RF-EMF effects for the subset of three species grown over the entire time of the experiment (see section 3.3). However, only the photographs of *Trifolium arvense* were finally retained and used in subsequent analyses. The photographs of *Viola arvensis* and *Avena fatua* taken three and four months after sowing were not fully reliable as the pixels that represented discolored leaf area blended in with the background pixels.

2.6.4. Leaf orientation analysis

We assessed RF-EMF impact on leaf orientation using visible plant leaf area observable from different viewpoints (recorded in photos taken on different perspective planes, see Fig. 3a). We used photos taken two months after sowing, when plants grew in multipot trays. By definition, leaf orientation is given by the direction of the normal (vector) of a leaf plane (Sinoquet and Andrieu, 1993) (Fig. 3b). Leaf orientation is hence defined by two angles: (1) the leaf inclination α, which is the angle between the leaf normal and the vertical axis, (2) the leaf azimuth Θ, *i.e.* the angle between the projection of the leaf normal onto the horizontal plane and a horizontal reference axis, such as the south direction (Sinoquet and Andrieu, 1993) (Fig. 3b). Based on this definition we created a leaf inclination index (i_α) and leaf azimuth index (i_Θ) and calculated them for each multipot tray (Appendix A). Then, for the sake of simplicity, we combined i_Θ, and i_α into one summary index: leaf orientation change index (i), using the rule of adding vectors in Cartesian coordinates:

$$i = \sqrt{{i_\alpha}^2 + {i_\theta}^2} \quad (1)$$

2.7. Statistical analysis

2.7.1. EMF effects in all 10 plant species two months after seeds sowing

For the first two months following seed sowing, the plants grew in multipot trays. These trays made up the sampling units in the experiment. An experimental group was made up by four multipot trays and we considered six response variables: seedling emergence rate r_e (%), plant height h (cm), plant shape index s, total leaf area la_t (cm^2), discolored leaf area la_d (%) and leaf orientation change index i. Data on dry aboveground biomass b was not used for statistical inference because it could be correlated with total leaf area or plant height. The analysis of RF-EMF impact on aboveground biomass is however presented in Appendix B. The variation in response variables was explained by two predictor variables: *treatment* and *species*. Factor *treatment* had two levels: radiation (r) or control (c), whereas factor *species* had 10 levels (10 plant species). The design was balanced with no factor combinations missing. The analysis was started by creating the plots of control vs. radiation mean, calculated for each species, one plot per response variable. The differences between these means were further investigated using an analysis of variance with planned contrasts incorporated into the models (Logan, 2010). ANOVA was performed for each of the response variables separately. To build the contrasts, two ANOVA factors: *treatment* and *species* were merged into one common factor named *group*, whose levels represented all possible combinations of *treatment* and *species* levels (Field et al., 2012). Before building contrasts, the values of an explained variable for individual species were scaled from 0 to 1 to stabilize variances and allow the comparisons of the possible RF-EMF effects among species. The following equation was used for scaling:

$$X_i = (x_i - x_{i,\min})/(x_{i,\max} - x_{i,\min}), \quad (2)$$

where X_i is the scaled value of a considered variable (height, shape index, etc.) of species i; x_i is a value of a considered variable of species i; $x_{i,\min}$ and $x_{i,\max}$ are the minimum and maximum values of a considered variable for species *i* (Czerwiński et al., 2018). One ANOVA covered 1200 plants growing in 80 trays (15 plants × 4 trays × 10 species × 2 variant plots). Normality and homogeneity of variance of the response variables were assessed by visual inspection of the boxplots of mean values (Logan, 2010). No obvious violations of these ANOVA assumptions were detected.

2.7.2. Temporal trends in green and discolored leaf area observed in *Trifolium arvense* plants exposed to electromagnetic field

Green and discolored leaf area (la_g and la_d) in *Trifolium arvense* were determined from the images taken at two, three and four months after sowing, i.e., one, two, and three months after germination of this species. These time series were used to investigate the changes occurring in the EMF-treated vs. control plants over the course of the experiment. The collected data represented a factorial model with two factors: *treatment* (radiation or control) and *time* (one, two or three months after germination). First, the means of la_g and la_d, averaged for the EMF-treated and control plants, were plotted against *time*. The size of the difference between the control vs. EMF treated plants was illustrated by error bars on the plots, calculated using between-subject (not within-subject) comparisons (Morey, 2008). Then model I ANOVA (with fixed effects) was applied to estimate general effects of *treatment* and *time*, as well as the interaction of these two factors. This interaction between *treatment* and *time* was further investigated using ANOVA with planned

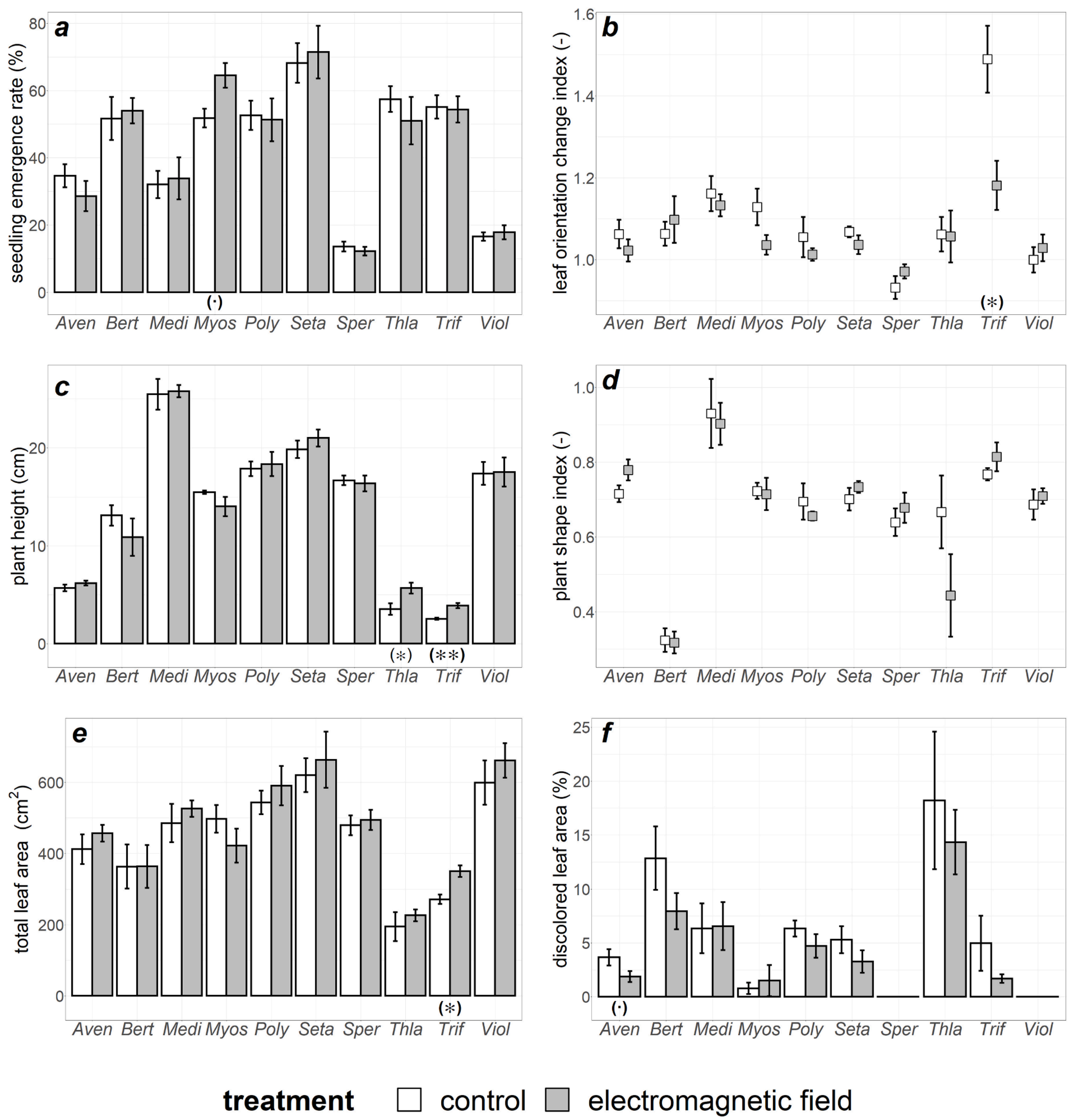

Fig. 5. The comparison between the EMF-treated vs. control plants for different plant characteristics: seedling emergence rate (a); leaf orientation change index (b); plant height (c); plant shape index (d); total leaf area (e); discolored leaf area (f); species names were shortened to save the space; the error bars represent standard error; the asterisks or dots above species names designate statistical significance of the difference between the irradiated and control plants ('**' designate 0.001 > p > 0.01, '*' designates 0.05 > p > 0.01,'·' designates 0.1 > p > 0.05); the significance was estimated using ANOVA with contrasts; data presented on figure (a) was collected almost one month after sowing, data presented on figures (b) – (f) was collected two months after sowing.

contrasts. To build the contrasts, two ANOVA factors: *treatment* and *time* were merged into one common factor named *group*, whose levels represented all possible combinations of *treatment* and *time* levels. The design was proportionally balanced: for the first month after seed germination, the experimental group (both EMF-treated and control) was represented by 60 plants, whereas for the second and third month after germination, the experimental group was represented by 24 plants. Due to this difference in group size, *time* was treated as a between-subject factor (Logan, 2010). Normality and homogeneity of variance of la_g and la_d were tested using the boxplots of mean values (Logan, 2010). There were no serious violations of these assumptions.

2.8. Presentation of the results, software used in the analysis

Significant differences between the control and EMF-treated plants detected in all analyses of variance were marked on the plots of the means using star or dot symbols. Detailed ANOVA results were presented in Appendix B. The entire analysis was performed in R, version 4.1.0 (R Core Team, 2021). R code provided by Mangiafico (2015) was applied to build the contrasts. Image processing and the calculation of *h* and *s* indices was performed using the "bwimage" R library (Biagolini-Jr and Macedo, 2020).

3. Results

3.1. General remarks

All species selected for this study germinated and reached maturity as normally developed plants: they reached their typical height and developed abundant foliage, with the majority producing flowers and seeds. Only *Avena fatua* and *Setaria viridis* did not enter the generative phase, while *Trifolium arvense* started to develop flowering shoots late in September. In June, before replacing the multipot trays with big pots, the plants probably suffered from sub-optimal growth conditions, as indicated by the appearance of reddish or yellowish leaf discolorations in most species, both in the radiation and control plot.

3.2. EMF effects in all 10 plant species two months after sowing

We found several differences between the EMF-treated and control plants in four out of ten species (Fig. 5). These differences related to different aspects of plant development and appeared at different stages, depending on the species. Thus, the seedling emergence rate r_e in *Myosotis arvensis* was higher in EMF-treated plants than in control plants by 12 %, $F_{1,19} = 3.54$, $p = 0.065$. The difference appeared much later in EMF-treated *Thlaspi arvense* plants that were higher by 60%, $F_{1,19} = 4.07$, $p = 0.048$ and in EMF-treated *Avena fatua* plants that had less discolored leaf area by 55%, $F_{1,19} = 3.07$, $p = 0.085$ (Fig. 5, Appendix B). The most significant differences were, however, observed in *Trifolium arvense:* plants exposed to RF-EMF were taller by 52%, $F_{1,19} = 8.25$, $p = 0.006$ and developed more leaf area by 24%, $F_{1,19} = 4.53$, $p = 0.038$ (Fig. 5, Appendix B). Qualitatively similar response was found for aboveground biomass (Appendix B). In addition, *Trifolium* plants had their leaves differently oriented, as indicated by *i*, $F_{1,19} = 4.59$, $p = 0.036$ (Fig. 5, Appendix B). This suggested changes in leaf orientation. Fig. 5f may also suggest that EMF-treated plants of *Trifolium arvense* had less reddish leaves, but it was not confirmed by ANOVA ($F_{1,19} = 1.98$, $p = 0.165$) due to high dispersion of la_d in the control plants.

3.3. Temporal changes in green and discolored leaf area in Trifolium arvense exposed to electromagnetic field

The response to RF-EMF detected in *Trifolium arvense* was analyzed using time series data on green leaf area and discolored leaf area at one, two and three months after germination. *Trifolium arvense* was the only species where this data for the three months was available. Two-way ANOVA performed to estimate general effects of the studied factors indicated that for both la_g and la_d the interaction between *treatment* and *time* is significant ($F_{1,5} = 12.69$, $p = 0.0005$ and $F_{1,5} = 9.86$, $p = 0.002$, respectively) (Table 1, Table 2).

The plots of temporal changes in green leaf area and ANOVA with planned contrasts (performed as a complement to the plots) showed that green leaf area was significantly larger in the EMF-treated plants compared to the control plants after one month, $F_{1,5} = 6.26$, $p = 0.013$ (Table B.2 in Appendix B, Fig. 6a below). However, this difference reversed when the plants grew bigger. Two months after germination, green leaf area was smaller in the EMF-treated plants, $F_{1,5} = 7.91$, $p = 0.005$, and three months after germination this this difference increased, $F_{1,5} = 8.88$, $p = 0.003$ (Table B.2 in Appendix B, Fig. 6a below).

The same analysis performed for the percentage of discolored leaf area in the EMF-treated plants vs. control plants showed the opposite trends. Discolored leaf area was significantly smaller in the EMF-treated plants compared to the control plants after one month of growth, $F_{1,5} = 6.31$, $p = 0.013$. However, in the second month of growth, la_d became much larger in the EMF-treated plants: $F_{1,5} = 6.36$, $p = 0.005$. In the third month this difference increased, $F_{1,5} = 17.89$, $p = 0.003$ (Table B.3 in Appendix B, Fig. 6b below).

The total leaf area (la_t) in *Trifolium arvense* over the three months of growth was different in EMF-treated vs. control plants. In the first month after seed germination, la_t was larger by 31% in EMF-treated plants (Fig. 5e). However, during the second month of growth, the la_t increase was greater in the control plants. As a result, in the third month la_t in the EMF-treated plants was smaller by 16% (not shown).

4. Discussion

4.1. EMF effects in all 10 plant species two months after sowing

Our measurements performed two months after sowing show that the RF-EMF exposure did not constitute a strong environmental signal for plants: no difference was detected between the EMF-treated and control plants for six out of the ten species tested (Fig. 5, Appendix B). Some differences were observed in *Myosotis arvense*, *Thlaspi arvense* and *Avena fatua*, but they occurred for only one response variable that differed according to the species: seedling emergence rate in *Myosotis*, plant height in *Thlaspi* and discolored leaf area in *Avena*, and the significance of these differences is small (*p*-value from 0.048 to 0.085).

However, a single species, *Trifolium arvense* was shown to have multiple traits that responded to exposure to RF-EMF. A significant difference between the control and EMF-treated plants was seen for plant height, total leaf area and leaf orientation change index (Fig. 5, Appendix B). The radiation-treated clover had their leaves differently oriented (in both the vertical and horizontal dimensions) and grew faster, which was reflected by increased total leaf area and plant height. *Trifolium arvense* plants were at the age of one month when all these effects were observed.

Table 1
The results of model I ANOVA to estimate general effects of treatment and time on green leaf area (cm^2) in the tested clover; the asterisks or dots designate statistical significance of the difference between the irradiated and control plants ('***' designate $0.0001 > p > 0.001$, '*' designate $0.001 > p > 0.01$).

	Degrees of freedom	Sum of squares	Mean square	F value	Pr(>F)
time	1	87,391	87,391	110.98	<2E−016***
treatment	1	395	395	0.50	0.480
time: treatment	1	9992	9992	12.69	0.0005***
Residuals	212	166,927	787		

Table 2
The results of model I ANOVA to estimate general effects of treatment and time on the percentage of discolored leaf area (cm2) in the tested clover; asterisks or dots indicate statistical significance, the same as in Table 1.

	Degrees of freedom	Sum of squares	Mean square	F value	Pr(>F)
time	1	7714	7714	93.47	<2E−016***
treatment	1	18	18	0.21	0.644
time: treatment	1	814	814	9.86	0.002**
Residuals	212	17,498	83		

4.2. Temporal changes in green and discolored leaf area in Trifolium arvense exposed to electromagnetic field.

Although the development of clover was faster in the first month for plants exposed to RF-EMF, as indicated by a larger height and leaf area (Fig. 5c and 5e or the first month after germination in Fig. 6), in the next months these plants became senescent earlier. This was manifested through the reduction of green leaf area, the concomitant increase of discolored leaf area, and smaller total leaf area developed over the growing season (Fig. 6, the second and third month after germination). Similar symptoms were recorded by Waldmann-Selsam et al. (2016) in trees around mobile phone base stations. Those trees were often characterized with sparse leaves, yellowish or brown leaf discoloring which started from the leaf margins, premature leaf fall and dead branches. In laboratory studies, the decrease of photosynthetic pigments in plants upon RF-EMF was reported by Sandu et al. (2005) for 400 MHz, Răcuciu and Miclăus (2015) for 1 GHz, and Stefi et al. (2017a, 2017b, 2017c, 2016) for 1882 MHz, who concluded that RF-EMF exposure induces stress in plants which leads to the reduction of the number of chloroplasts in leaves, as well as to structural damages and pigment reduction in these chloroplasts.

The observed long-term response of *Trifolium arvense* to RF-EMF can be interpreted through the prism of plant capability for adaptation to stress resulting from exposure to RF-EMF. A set of molecular responses have been attributed to RF-EMF and may increase plant resistance to radiation-induced stress (Beaubois et al., 2007; Kundu et al., 2021; Roux et al., 2008, 2006; Vian et al., 2006). In line with these findings, Halgamuge (2017) hypothesized that plants, due to their metabolic and phenotypic plasticity, can adapt to continuous RF-EMF exposure. In our study these adaptations were not sufficient for *Trifolium arvense* to cope with RF-EMF stress, because it ultimately led to premature death of the individuals of this species. The sensitivity of *Trifolium arvense* to RF-EMF might have been increased by the influence of natural environmental stressors, such as water deficit, strong winds and rains, etc. This hypothesis is in line with the results by Tran et al. (2023) who found out that plant tolerance to RF-EMF-induced stress is reduced when the plant grows outdoors and is subjected to a variety of natural environmental stressors.

4.3. Plant response to RF-EMF: trait-level analysis

Our analysis has shown that two months after sowing seeds, EMF-treated plants of *Thlaspi arvense* and *Trifolium arvense* were of a greater height than the control plants, with the latter also having a larger total leaf area. This effect was stronger for height than for total leaf area, because the effect size is statistically greater, as indicated by F values, and it refers to both *Trifolium* and *Thlaspi*. Changes in height have been probably the most often reported morphogenetic alteration in plants exposed to man-made RF-EMFs (Kaur et al., 2021; Vian et al., 2016). In the majority of these studies, plants exposed to RF-EMF were of smaller height than the control plants. In the studies where height increased, stem extension was observed by Jinapang et al. (2010), Răcuciu and Miclăus (2007) and Mildaziene et al. (2016), while both stem and root extension was observed by Surducan et al. (2020). The increase of height and leaf area could be a direct consequence of the intensification of photosynthetic activity upon RF-EMF but the evidence that supports such an explanation is scant (Kaur et al., 2021; Răcuciu and Miclăus, 2007). Another possible explanation could be that RF-EMF stimulated these changes indirectly, through the impact on leaf orientation (see below). Nonetheless, these outcomes confirm the hypothesis that plant canopy height can be a sensitive indicator of environmental RF-EMF effects (Czerwiński et al., 2020). This is particularly the case when electromagnetic waves are vertically polarized and fall at an acute angle to the ground (a common RF-EMF propagation scenario in the environment). Where this configuration occurs the absorption of EMF energy by erect stems or vertically oriented leaves can be particularly high (Gómez et al., 2011).

It is worth discussing why RF-EMF driven increase in height co-occurred with the increase in total leaf area in *Trifolium arvense,* but not in *Thlaspi arvense*. It might be explained by clover's symbiosis with

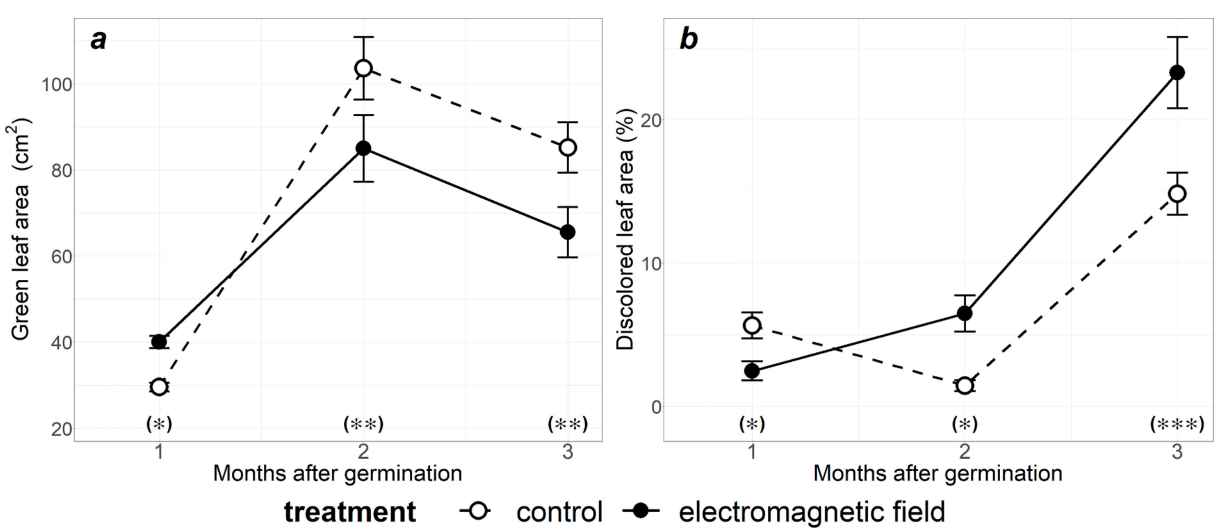

Fig. 6. Temporal changes in green (a) and discolored leaf area (b) in Trifolium arvense exposed to RF-EMF; the error bars represent standard error; the asterisks or dots above species names designate statistical significance of the difference between the irradiated and control plants (the symbols and significance levels are the same as in the previous figures); the significance was estimated using ANOVA with contrasts.

both mycorrhizal fungi and *Rhizobium* bacteria, which provide additional resources of nitrogen and phosphorus to the plant (Kuyper and de Goede, 2013). These resources could mitigate nutrient deficits that probably occurred in June, allowing for a normal foliage development. *Thlaspi arvense*, which does not host these plant microbial and fungi symbionts, grew higher in June, but developed foliage relatively slowly, especially in the upper half of the plant. This difference is expressed by shape difference between *Trifolium* and *Thlaspi* (Fig. 5d).

EMF caused changes in the orientation of leaves in one species, *Trifolium arvense*. This species, as well as another studied species, *Medicago falcata*, can orientate their leaves in response to the angle of the incident solar radiation or leaf temperature to optimize photosynthetic activity (Ehleringer and Forseth, 1980, 1989). It may be expected that leaf orientation changes in *Trifolium* led to a reduction of light interception in this light-demanding species. A consequence of the reduced light interception might be that plants invested a disproportionate amount of biomass to increase height and leaf area. Such a phenomenon has been documented as a response to shading (Bazzaz, 1996; Hutchings and de Kroon, 1994) and is in agreement with the other results of our study: increase of height and total leaf area in *Trifolium*.

To summarize this part of discussion, we have drawn a scheme that briefly illustrates both our empirical findings and the hypothetical background of RF-EMF impact (Appendix C).

4.4. Possible ecological implications at plant community or ecosystem scale

In our experiment plant response to RF-EMF was largely undetectable or restricted to a single trait for most of the considered species. Only for one of the 10 species, *Trifolium arvense*, were there distinct effects. This suggests that possible consequences of environmental exposure to man-made RF-EMFs are also restricted to specific plant species. Our study does not answer to the question as to whether RF-EMF exposure from radiocommunication base stations drives changes that affect wider trophic interactions in ecosystems as *Trifolium arvense* does not compose large proportions of common plant communities in Europe. This is especially so when compared to other *Trifolium* species, such as *T. pratense* or *T. repens* (Leuschner and Ellenberg, 2017). However, our results lead to an important question as to whether the response observed in *Trifolium arvense* is specific to this species or it refers to that of other taxonomically closely related species. Numerous *Fabaceae* species have been reported to be sensitive to RF-EMF at non-thermal exposure levels. This includes *Glycyne* max (Halgamuge et al., 2015), *Cicer arietinum* (Qureshi et al., 2017), *Vigna radiata* (Sharma et al., 2009; Singh et al., 2012), *Phaseolus vulgaris* (Surducan et al., 2020), *Robinia pseudoaccacia* (Waldmann-Selsam et al., 2016) and *Lens culinaris* (Akbal et al., 2012). It is also possible that the response observed in *Trifolium arvense* is a common feature of other species within the genus *Trifolium. If so* some types of RF-EMFs used for telecommunication may exert noticeable impacts on the functioning of entire grassland communities, especially in urban or suburban areas. *Trifolium spp*., like other *Fabaceae*, are distinguished by the ability to symbiotically fix atmospheric nitrogen, and this is one of the major functional traits of plants because they improve the use of available N resources in the ecosystem, facilitate the growth of plants from other families (which take up the fixed N) and have a positive effect on the decomposition of organic substrate (Spehn et al., 2005). Among different *Fabaceae* genera, *Trifolium* is considered a keystone component within European grasslands, because these plants are particularly efficient N_2-fixers (Brun et al., 2022; Spehn et al., 2002).

5. Conclusions

To the best of our knowledge, our study is the first attempt to assess RF-EMF impact on different wild plant species in an experiment conducted in open natural environment where the control conditions are carefully established. Our findings clearly show that none of the hypotheses formulated in our research can be rejected, at least for some species. We observed RF-EMF effects in plants exposed to natural environmental stresses (not growing in optimum laboratory conditions) and these effects were permanent and irreversible. Furthermore, for some plant species the response to RF-EMF was clear, whereas for the others it was weak and difficult to detect, or showed no response at all. For one species, *Trifolium arvense*, RF-EMF occurred at different plant development stages and for different plant characteristics, which were measured using different methods, and all these effects seem to be reciprocally consistent. Although our study does not provide an unambiguous answer to whether the development of wireless technologies has serious ecological implications for wild plant communities, it is a step forward in the research on this problem. We conclude that future studies should focus on *Trifolium* species or other legumes that underpin common grassland ecosystems. Our findings also suggest that *Trifolium arvense* can be considered a candidate for the indicator of ecological effects of man-made EMFs in the environment.

CRediT authorship contribution statement

Marek Czerwiński: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Alain Vian:** Conceptualization, Methodology, Writing – review & editing, Visualization. **Ben A. Woodcock:** Writing – review & editing. **Piotr Goliński:** Writing – review & editing, Funding acquisition. **Laura Recuero Virto:** Writing – review & editing. **Łukasz Januszkiewicz:** Conceptualization, Methodology, Software, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization.

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

We thank Iwo Gałecki and Roman Malik who provided access to the research area and hosted the experiment. Roman Malik helped us also with the set-up of the study, gave us many constructive comments and provided his assistance in the field. We are also grateful to Agnieszka Jaros for valuable discussion and advice on statistical analysis.

This study was funded by the National Science Centre Poland (NCN), under research project "The impact of electromagnetic fields used in wireless communication on the growth and development of herbaceous plants" no 2022/04/X/NZ8/01792. Publication was co-financed within the framework of the Polish Ministry of Science and Higher Education's program: "Regional Excellence Initiative" in the years 2019–2023 (No. 005/RID/2018/19).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110267.

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