Impact of mechanical mowing and chemical treatment on phytosociological, pedochemical and biological parameters in roadside soils and vegetation

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Abstract Many chemical and non-chemical strategies have been applied to control weeds in agricultural and industrial areas. Knowledge regarding the effects of these methods on roadside vegetation is still poor. A two-year field experiment was performed along a road located near Livorno (Tuscany, central Italy). Eight plots/strips were identified, of which four were subjected to periodical mechanical mowing and the remaining four were treated with a chemical herbicide based on glyphosate (the producer's recommended rates were used for the selective control of broad-leaved weeds). Our results clearly showed that roadside soil and vegetation are a significant reservoir of anthropogenic activities which have a strong negative effect on several phytosociological, pedochemical and biological parameters. Compared with conventional mechanical mowing, chemical treatment induced (i) a significant increase in organic matter in the upper plot layers (+18%), and (ii) a marked reduction in weed height throughout the entire period of the experiment. Irrespectively of the kind of treatment, no significance differences were detected in terms of (i) biological quality of soil (the abundance and diversity of arthropod communities did not change), and (ii) plant elemental content (bulk concentrations of analysed trace elements had a good fit within ranges of occurrence in the "reference plant"). The glyphosate partially controlled broad-leaved weeds and this moderate efficacy is dependent upon the season/time of application. In conclusion, the rational and sustainable use of chemical herbicides may be a useful tool for the management of roadside vegetation.

Keywords enrichment factor, glyphosate, herbicide, trace elements, weeds, weed control

Introduction

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Dramatic ecological events take place near alongside roads (Forman and Alexander 1998). Road networks interrupt horizontal ecological flows, fragment habitats, alter landscape spatial patterns. Faunal casualties (not only vertebrates) are very common, and traffic noise is a disturbing element. In addition, road traffic impacts on air quality and is one of the main anthropogenic emission sectors for nitrogen oxide (NOx), carbon monoxide (CO) and non-methane hydrocarbons (NMHCs). In the atmosphere, these compounds act as ozone precursors, by forming radicals that eventually contribute to ozone (e.g. Nali et al. 2001) and secondary organic aerosol (such as volatile organic compounds, VOCs, e.g. Pellegrini et al. 2012) formation.

A series of substances emitted by vehicles have been qualified as toxic. The US EPA highlights 21 toxic chemical species that can mainly be assigned to road traffic, including several heavy metals (e.g. Pb, Cd, Cr, Sb, Cu, Zn, Ni). Roads with heavy traffic have shown elevated concentrations of such elements at distances of up to 1000 m (Zechmeister et al. 2005). The metals introduced into the environment by traffic derive from many sources: tyre wear off; break lining; wear of vehicular components such as the car body, clutch or motor parts; and exhaust fumes. Pollutants released by motor vehicles may also originate from the residues from incomplete fuel combustion, oil leaking from engines and hydraulic systems, and fuel additives (Thorpe and Harrison 2008). Moreover, road abrasion, pavement leaching, road maintenance (e.g. de-icing) are also significant sources of pollution (Werkenthin et al. 2014).

Brake dust has been recognized as a significant carrier of Cu and Sb in the composition of aerosol (Sternbeck et al. 2002). In fact, Cu is used in brakes to control heat transport, and Sb to enhance stability. Ba is used as $BaSO_4$ to increase the density of brake pads (Sternbeck et al. 2002). Zn is significantly prevalent in tyres, as ZnO is added as an activator and a preservative during the vulcanizing process and approximately 1.8% of the tyre weight is Zn (Pierson and Brachaczek 1974).

The corrosion of galvanised safety barriers and signs may be another source of Zn (Helmreich et al. 2010). The road surface itself is an important source of pollutants, since the components of asphalt, i.e. stone material and bituminous binders release several contaminants (Mangani et al. 2005). In addition, the main active components of present-day car catalysts consist of the noble metals Pt, Pd and Rh, which are being diffused into the environment to an as-yet unknown extent (Palacios et al. 2000). Metals cannot be decomposed by micro-organisms and have a long-term toxicity for biota and a massive negative impact on the environment due to their potential for bioaccumulation. In addition to the above mentioned chronic sources of pollution, temporary (e.g. road works), seasonal (de-icing, salting) and accidental (hazardous chemicals) sources may play an impact on the roadside environment.

Adjacent to a road there is often a land strip, usually dominated by herbaceous vegetation. Plants on this strip tend to grow rapidly with ample light and with moisture from road drainage. Thus, periodical management of this vegetation is time (and money) consuming. There are two major strategies to manage roadside vegetation: mechanical mowing and herbicide application. Both have pros and cons, such as:

- ✓ Effectiveness: mechanical cutting stimulates the continuous production of new biomass. "*The more you cut a plant, the more it reacts and produces new leaves and stems*", thus several cuts need to be performed in a year.
- ✓ The management of plant residues: this is a difficult task, however the long-term presence of residues may be a problem for regular water drainage.
- ✓ Risks for operators, due to vibration, noise, chemical pollution, projectiles (stones) during mowing, and chemical pollution during herbicide distribution.
- ✓ Ecological impact with mowing, especially on animal biodiversity; risks of environmental pollution with chemical treatments.

Other key issues concern logistics (roadworks involve traffic restrictions and financial costs). Clear management strategies are required to reduce the number of maintenance services: a final goal in terms of best management practices should be the attainment of a permanent, low profile, phytocenosis dominated by particular evergreen creeping species, so to reduce the running costs.

Glyphosate (N-[phosphonomethyl]glycine) is a broad-spectrum herbicide, used non-selectively in agriculture to control weeds and herbaceous plants (Duke and Powles 2008). Since 1970, it has become one of the most widely used herbicides worldwide (Van Stempvoort et al. 2014). Though its major uses are in agriculture and silviculture (along roadways, railways and utility corridors), it has recently been used to control weeds in urban and residential areas (streets, sidewalks, paved areas, gardens) in many European countries (Botta et al. 2009). It works by inhibiting the enzyme 3-enol-pyruvylshikimate-5-phosphate synthase (EPSP synthase), located in the chloroplast, thus interfering in the biosynthesis of aromatic amino acids used in the synthesis of proteins. This enzyme forms part of the metabolic pathway of the shikimic acid. This process only occurs in plants, bacteria and fungi, and does not exist in animals. As a consequence acute toxicity in animals is regarded as very low (Giesy et al. 2000), although there is some contradictory evidence about glyphosate toxicity (e.g. Zhou et al. 2012; Cuhra et al. 2013).

The majority of investigations into glyphosate use in urban areas have focused on the quantification and characterization of herbicide loss, especially of aminomethylphosphonic acid [an important metabolite produced during microbial degradation of glyphosate (e.g. Borggaard and Gimsing 2008)], in surface water and groundwater (Tang et al. 2015). Few field studies have investigated the sustainable use of this herbicide on roadsides.

The aims of this two-year field study were to evaluate the impact of two common management strategies of roadside vegetation (i.e. mechanical mowing and chemical treatment with the chemical herbicide glyphosate) on several biological soil and vegetational parameters and on the ability of plants to intercept the metal pollution arising from road traffic.

Material and methods

Field investigations

The trial was conducted at a section (Lat. 43°33'47" N – Long. 10°21'38" E, 11 m a.s.l.) of the Livorno-Cisternino dual carriageway road, Via delle Sorgenti, located near Livorno (Tuscany, Central Italy). The average bidirectional traffic volume is around 10,000 vehicles day^{-1}. The speed limit is 70 km h^{-1}. The road is slightly bendy and the main land use surrounding the sampling sites is grassland and forest areas. Mean annual daily maximum temperature is 19.0 °C (range 10.8 °C, January - 27.7 °C, July); mean annual daily minimum temperature is 12.1 °C (range 4.8 °C - 20.0 °C); the average annual precipitation is approximately 760 mm, spread over about 85 rainy days.

Eight plots/strips [each test plot was 75 m^2 (1.5 m width, 50 m length)] were selected: four of which were treated according to the usual method adopted by the local authorities (e.g. periodical mechanical mowing); the remaining four were treated with a commercial formula (*Rodeo Gold*, Monsanto Europe – Antwerp, Belgium) containing 41% of glyphosate in water. In order to control broad-leaved weeds and to maintain acceptable annual grass weed levels, the recommended field concentration of *Rodeo Gold* (5.3 L of active ingredient ha^{-1}) was used. The dosage was 40 mL formulate diluted in 1.5 L of water for each plot. No coformulant or additive was added. Soil texture proved to be homogeneous amongst the plots: the vertical profile of the loamy sand was between 0-10 cm and the sandy loam profile was between 10-30 cm. The chrono program of phytosociological, pedochemical and biological sampling/analyses was: 20 January, 2011 (T_0); 16/18 May, 2011 (T_1), 22 February, 2012 (T_2), 3-5 July, 2012 (T_3) and 7 November, 2012 (T_4). At T_0 and T_4, plant sampling was carried out for the elemental analysis. After the environmental characterization of soils and vegetation communities, the mowing/chemical treatments were carried out. The chrono program of treatments was: 20 January, 2011; 16/18 May, 2011; 1 August, 2011; 22 February, 2012; 3-5 July, 2012 and 7 November, 2012.

Samplings and analyses

Soil

At each sampling time, using a manual soil probe (5 cm diameter), triplicate soil cores were taken at each of two depths (0-10 and 10-30 cm) from random positions across each plot for the determination of (i) soil pH (Mc Lean 1982), (ii) electrical conductivity (EC) by a conductivity meter (Wilcox 1947), (iii) organic matter (Nelson and Sommers 1982), (iv) assimilable P (Olsen and Sommers 1982), (*v*) total N (Brement and Mulvaney 1982).

Arthropod community evaluation

Three 50-cm deep dugouts were randomly digged in each plot. The arthropod extraction was performed separately for each of the soil cores. The samples were carefully placed on a 1.5 mm mesh net above Berlese-Tullgren funnels for seven days; the extracted material was preserved in 95% ethanol+1% glycerol. This method, widely used in ecological studies of soil arthropods (Macfadyen 1961), allows for the extraction of mites (*Acari*), springtails (*Collembola*), and arthropods with a dimension <1.5 mm that are present in the soil samples. Arthropods were counted, identified and classified on a taxonomic level not lower than order. The biological quality index of soil based on microarthropods (QBS-ar) (Parisi et al. 2005) was computed. This is not dependent on species identification, but considers arthropods grouped with a more general ordering. The *Acari/Collembola* ratio was also computed, which is a further biological index of environmental quality, as mites are considered much more susceptible to chemical stress than springtails (Paoletti et al. 1991; Huguier et al. 2015).

Plant biodiversity indices

The differences in biodiversity were recorded using the Braun-Blanquet phytosociological system, which is a rapid, visual assessment technique (Braun-Blanquet 1964). This method requires only minutes at each sampling site, yet it is robust and highly repeatable, thereby minimizing amongobserver differences. At each plot, 15 to 27 random throwings of a 25x35 cm rectangle made out of steel wire were used to select the monitoring sites, each of which were then examined. All the plant species in the rectangle were listed, and a score based on the cover of the species in that area was assigned: score 5: cover 80-100%; 4: 60-80%; 3: 40-60%; 2: 20-40%; 1: 1-20%; +: <1%, but several individuals are present; r: <1%, very few individuals are present. Cover, as defined for this purpose, is the fraction of the total rectangle area that is obscured by a particular species when viewed directly from above. The frequency of each species was determined according to the following scores: score V: frequency 80-100%; IV: 60-80%; III: 40-60%; II: 20-40%; I: 1-20%.

The following ecological indices were then computed according to the biodiversity calculator (http://www.alyoung.com/labs/biodiversity_calculator.html):

(a) *Shannon*: a diversity logarithmic index (H') that accounts for both abundance and evenness of the species present. It is explained by the formula: $H' = -\sum (Pi * \ln Pi)$, where: H' = the Shannon diversity index; Pi = fraction of the entire population made up of species i (proportion of a species i relative to total number of species present). Here, a high value of H' is representative of a diverse and equally distributed community, and lower values represent a less diverse community. A value of 0 represents a community with just one species.

(b) *Simpson*: the dominance index (D), a simple mathematical measure that characterizes species diversity in a community (Simpson, 1949). It equals $D = \Sigma (n/N)^2$, where: n = the total number of organisms of a particular species; N = the total number of organisms of all species. The value of D ranges between 0 and 1, where 1 represents infinite diversity and 0 no diversity.

(c) *Equipartition*: this indicator (E) describes to what extent a community approaches the ideal case of the equipartition of individuals amongst the species, E = H'/Hmax, where: H' = Shannon index; Hmax = Shannon index computed for a theoretical biocenosis, where all taxa present exactly the same number of individuals. This value is the maximum when all the species are present with the same abundance, and is minimum when only one species is abundant and the others are rare.

Plant height

Grass height was measured by a home-made herbometer on randomly selected subplots.

Plant elemental analysis

Total concentrations of Al, As, Ba, Cd, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Na, Ni, Pb, Sb, V and Zn in washed and unwashed plant samples were determined (Pellegrini et al. 2014). Three composite samples representing above-ground parts were randomly taken from each plot; half were treated as collected ('unwashed'), the other half were washed with distilled water ('washed'). The samples were oven-dried at 80 °C for 24 h and ground into a powder. About 10 g of powder were mineralized with a 6:1 (v:v) mixture of ultrapure concentrated HNO_3 and H_2O_2 at 280 °C and a pressure of 0.55 MPa in a microwave digestion system (Milestone Ethos 900). Element concentrations, expressed on a dry weight basis, were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP–MS, Perkin Elmer–Sciex Elan 6100). Analytical quality was checked with the Certified Reference Material GBW07603. All analyses were carried out in triplicate. Accuracy was within 7% for all elements.

Statistical treatment of data

All data referred to pairs of values were processed by means of Student's t-test. To get an indication of the relative contribution of crustal contamination to the bulk element distribution in and on the plant material, enrichment factors (EF) were calculated for each element, taking Al as a reference element. The average crustal composition reported by Taylor and McLennan (1985) was used. The dimensionless EF for any element X relative to crustal material is defined by $EF=(X/Al)_{leaf}/(X/Al)_{crust}$, where: $(X/Al)_{leaf}$ is the concentration ratio of X to Al in the plant sample; $(X/Al)_{crust}$ is the average concentration ratio of X to Al in the crust. The elemental composition of unwashed leaves was subjected to Varimax rotated factor analysis (FA), which is a multivariate statistical treatment frequently used in atmospheric pollution research to obtain information on pollution sources (Lorenzini et al. 2006). NCSS 2004 SAS software was used.

Results and discussion

Soil chemistry

As reported in Table 1, soils were moderately alkaline and mean pH values of vertical profiles were similar between mowed and chemically treated plots. At the beginning of the trial, mean EC values of vertical profiles were similar between both plots that were to be mowed and those subjected to chemical treatments. This parameter was not affected by treatments at the end of the experiment (Table 1). At the beginning of the experiment, organic matter percentages of vertical profiles were very similar between both plots. The deeper profile was consistently lower in organic matter than the superficial profile, regardless of treatment or period of sampling. Organic matter content significantly decreased at the end of the test, irrespectively of the treatment.

According to Baboo et al. (2013), this result suggests that (i) transformations of nutrients, and (ii) alterations in the soil performance can occur as a consequence of mechanical mowing and/or herbicide application. The upper layers of the plots treated with the chemical were also significantly richer in organic matter than the counterparts subjected to mechanical mowing. Similar results were documented by Sebiomo et al. (2011).

At the beginning of the experiment, P and N contents of vertical profiles were very similar amongst the plots. The deeper profile was lower in nutrient concentrations than the superficial profile, regardless of treatment or period of sampling. Regarding N, nitrogen oxides emitted by vehicle exhausts and absorbed by roadside soil were likely to be the cause of these effects (Spencer and Port 1988). At the end of the experiment, no statistically significant difference was observed amongst plots treated with the chemical herbicide or mowed, which was the case for both vertical profiles investigated.

Arthropod community evaluation

A total of 11 microarthropod taxa were recorded (data not shown) and used to estimate the biological quality index of the soil (QBS-ar), which was regularly assessed during the five sampling periods. As this index may vary from 0 to a maximum of 349 (Parisi et al. 2005), our data present extremely low values (Table 2). No significant differences were also observed throughout the entire period of the experiment, irrespectively of the kind of treatment.

The variability in soil biota abundance and diversity is influenced by many factors that are only partially understood, such as (i) soil properties, (ii) landscape characteristics, (iii) management practices, (iv) interaction with aboveground biodiversity, and (v) seasonality. In agreement with Aspetti et al. (2010), the yearly fluctuation in QBS-ar values was smaller than the monthly variation caused by seasonal climate effects. Multiple samplings within short distances or time periods (40-86 days) reproduced similar QBS-ar values. For each sampling time, none of the differences between treatments proved to be statistically significant in terms of QBS-ar values. This suggests that (i) no alterations in soil microarthropod biodiversity occurred as a consequence of treatments, and (ii) the selected/used concentration of *Rodeo Gold* had no effect on the scarce biological quality of the soils. In agreement with Blasi et al. (2013) and Galli et al. (2014), *Acari* and *Collembola* were present in all the plots and their ratio was very low (even below 1). No significant differences were detected between the two kinds of treatment (data not shown), which is a further confirmation of previous findings. Studies investigating the effect of *Rodeo Gold* on biological quality of soil are scanty and, generally, are carried out in experimental agricultural fields (Cherni et al. 2015).

Plant biodiversity indices

As reported in Table 3, selected plant biodiversity indices showed that no significant difference was observed between plots, regardless of treatment. Low values of the Shannon index (about 1) were observed throughout the entire period of the experiment, suggesting that the abundance and the evenness of the species were very scanty. In agreement with Wu et al. (2015), this is representative of a less diverse and not equally distributed community. An estimation of the Simpson index also revealed a dominancy of few species (Giliba et al. 2011), suggesting that a marked anthropogenic pressure (regardless of treatment) affected the species diversity in this community. In relation to the Equipartition index, low values observed at the beginning and at the end of the treatment showed that this community came nowhere near the ideal equipartition of individuals amongst species. In fact, the general situation of plots was that only a single species was abundant and others were rare. Similar conclusions were reported by Dewar and Porté (2008). At the end of the experiment, E values showed a slight increase in comparison with T_0 (regardless of the treatment). Most of these observations are in accordance with field studies on roadside vegetation outside Italy (Cillieris and Bredenkamp 2000).

Table 4 describes the phytosociological profiles of eight plots at T_0, T_2 and T_4. During and at the end of the experiment (in winter), the chemical treatment had a differential efficacy on individual broad-leaved weeds. The tested formulation of glyphosate completely controlled *Daucus carota*, *Fumaria* and *Potentilla repens* throughout the experiment. By contrast, *Rodeo Gold* produced the highest controlling rates against *Anagallis*, *Bellis*, *Plantago*, *Silene*, *Sonchus* and *Trifolium* at T_2. At T_4, frequencies of genus *Capsella*, *Equisetum*, *Euphorbia*, *Geranium*, *Senecium* and *Veronica* were reduced in comparison with T_2, suggesting that the efficiency of selected/used dosage of the formula varied among treatment times. Similar findings were reported by El-Kholy et al. (2013), who demonstrated that the efficacy of chemical treatment against broad-leaved weeds is dependent upon the (i) weed species and (ii) season/time of application.

Mowing produced the lowest controlling rates against *Equisetum*, *Rubus*, *Senecium*, *Trifolium* at T_4, suggesting that this treatment may create a new competitive environment. In addition, frequencies of genus/species *Euphorbia*, *Galium*, *Potentilla repens*, *Rumex* and *Trifolium* increased in comparison with T_0, confirming that the abundance of some weeds may significantly change as a consequence of mowing (in agreement with Busey 2003).

Plant height

Fig. 1 shows how grass heights were constantly higher in plots subjected to mechanical mowing compared to those treated with the chemical herbicide. Mowing can be considered as a mechanical stress that differentially affects weed communities. For certain weeds, cutting removes part of the photosynthesizing tissues and, consequently, leads to the exhaustion of carbohydrate reserves during regrowth (Fry and Huang 2004). In other cases, especially for some perennial weeds that replenish root carbohydrate stores very rapidly (Foster 1989, Donald 1990), cutting can be advantageous by (i) removing old/dead tissues, (ii) branching or tillering after release from apical dominance, (iii) increasing new shoots and relative growth rates (Strauss and Agrawal 1999). In agreement with Abu-Dieyeh and Watson (2005), our results suggest that mechanical mowing may disturb the competitive relationship between interacting species, thus encouraging the growth and development of certain species.

Plant elemental analysis

At the beginning of the experiment (Table 5), bulk concentrations of Ba, Co, Cr, Fe, Mn, Ni, Pb, Sb and V in/on samples (regardless of washing) were much higher than the "reference plant" and very near toxicological standards (Markert 1992). This is probably because several sources of pollution play a pivotal role in our study area. This result is further confirmation of previous findings regarding trace metal contamination in roadside vegetation and/or soil (Zechmeister et al. 2005; Aslam et al. 2013; Modrzewska and Wyszkowski 2014). Regardless of the treatment and washing (unwashed *vs* washed material), concentrations of all 17 selected elements (except Mg) showed a significant decrease at the end of the experiment. The abundant rain that characterized the last days of October and the first days of November 2012 could have removed the (i) surface airborne dust, (ii) adsorbed material and (iii) any soil particles deposited on the samples. Our plant data sampled at end of the experiment have a good fit within ranges in higher plants proposed by Markert (1992). Only Ba and Sb had mean values slightly higher than those reported for the "reference plant".

For an indication of the relative contribution of crustal contamination to the mass of element distribution in plant tissues, EFs were calculated for nine elements (Ba, Co, Cr, Fe, Mn, Ni, Pb, Sb and V) in unwashed samples which at the beginning of the experiment, showed very high bulk concentrations (compared to the "reference plant"). The most enriched element was Ni, with EFs values up to 10^2 (Table 6), which indicates a heavy contribution of non-crustal sources (Duce et al. 1975). Ba, Co, Cr, Fe, Pb, Sb and V were considered as enriched with EFs in the range 10-100. EFs down to 10 (observed for Mn) may be considered as samples that were not significantly enriched, due to differences in the chemical composition of local soil and the reference crustal composition. The same analyses were performed at the end of the experiment, taking into account the kind of treatments. No statistically significant difference was observed amongst the EF values obtained with the initial and the final samplings, regardless of treatment.

FA with Varimax rotation was applied to the dataset of total concentrations, with 10 elements included in the analysis. At the beginning of the experiment (Table 7, above), just two interpretable factors explained almost the entire total variance. The first factor, characterized by significant loadings for Ba, Co, Cr, Ni, Pb, Sb, and V, can be easily identified as a predominant vehicular traffic contribution and accounts for 61.2% and 59.7% of total variance (for mowing and chemical treatment, respectively). The second factor is loaded with Al, Fe and Mn, which could be related to the natural origin. Key loading elements in the "vehicular traffic" factor are Ba, Pb, Sb and Zn, which have long been indicated as traffic-related elements (TREs) and suitable indicators for motor vehicle related emissions (Fujiwara et al. 2011).

It is interesting how Pb still represents significant pollution in the study area, although since 2002, Italy has completely phased out petrol that contains lead tetraethyl, by switching to alternative lead-free antiknock additives.

Ba has many applications in the automotive industry and is currently regarded as the best inorganic tracer of vehicular traffic (see e.g. Sternbeck et al. 2002). Sb is ubiquitously present in the environment as a result of natural processes and human activities. In recent years, Sb has been associated with traffic and identified as a TRE (Paoli et al. 2012): several parts of vehicles contain Sb alloys and other Sb compounds. In relation to its toxic properties and its dispersion in the environment, Sb has been the focus of many pollution studies (e.g. Guéguen et al. 2012). A key loading element in "crustal (soil) contamination" factor is Al, considering its (i) limited metabolic significance in plants and (ii) wide distribution in the Earth's crust (Taylor and McLennan 1985).

The same analyses were performed at the end of the experiment (Table 7, below), taking into account the kind of treatments. In plots subjected to mechanical mowing, two factors explained 98.3% of the total variance. The first factor can be easily linked to vehicular traffic, characterized by high loadings for Ba, Cr, Ni, Pb, Sb and V. The second factor is loaded with Al, Fe and Mn, and could be related to crustal (soil) contamination. In terms of data related to plots subjected to the chemical herbicide, very similar data were obtained.

Conclusions

A vital element roadside maintenance is vegetation care. Strips of natural vegetation have a number of functions and act as buffers between traffic and the rest of the landscape, absorbing noise, dust and water from road surfaces. However, roadside vegetation management is critical, expensive and time-consuming. Maintaining roadsides for safety and aesthetics is an important issue at all levels of government. Desirable vegetation is a fundamental element of roadside maintenance and landscape promotion.

The two-year long field experiment described here highlights how the rational and sustainable use of a chemical herbicide based on glyphosate could be a useful approach to the management of wild and volunteer roadside vegetation. Compared with conventional mechanical mowing, this approach of precision farming could ensure the constant presence of low-height and stable grass covering, without any negative disturbance in terms of the chemical and biological features of the soil. Also significant is the financial perspective, as chemical treatment is far cheaper than mechanical mowing.

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Ethical approval “All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.” This research adheres to the ASAB/ABS Guidelines for the Use of Animals in Research (2012). All treatments of the experimental animals (acari and collembola) were in compliance with Italian regulations on the protection of animals used for experimental and other scientific purposes (D.M. 116192). All experimental procedures also followed the animal care guidelines of the University of Pisa Ethical Committee. No particular permits were needed from the Italian government for experiments involving acari and collembola.

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Caption of figure

Fig. 1 Average grass heights during the experiment. Mean values ±standard deviations are shown. For each sampling date, asterisks indicate the significance of the means between treatments (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, Student's-test). Arrows show treatments (black arrows: chemical herbicide; white arrow: mechanical mowing). A: January 10 - May 15, 2011; B: May 16, 2011 – February 20, 2012; C: February 20 - July 5, 2012; D: July 6 - November 7, 2012

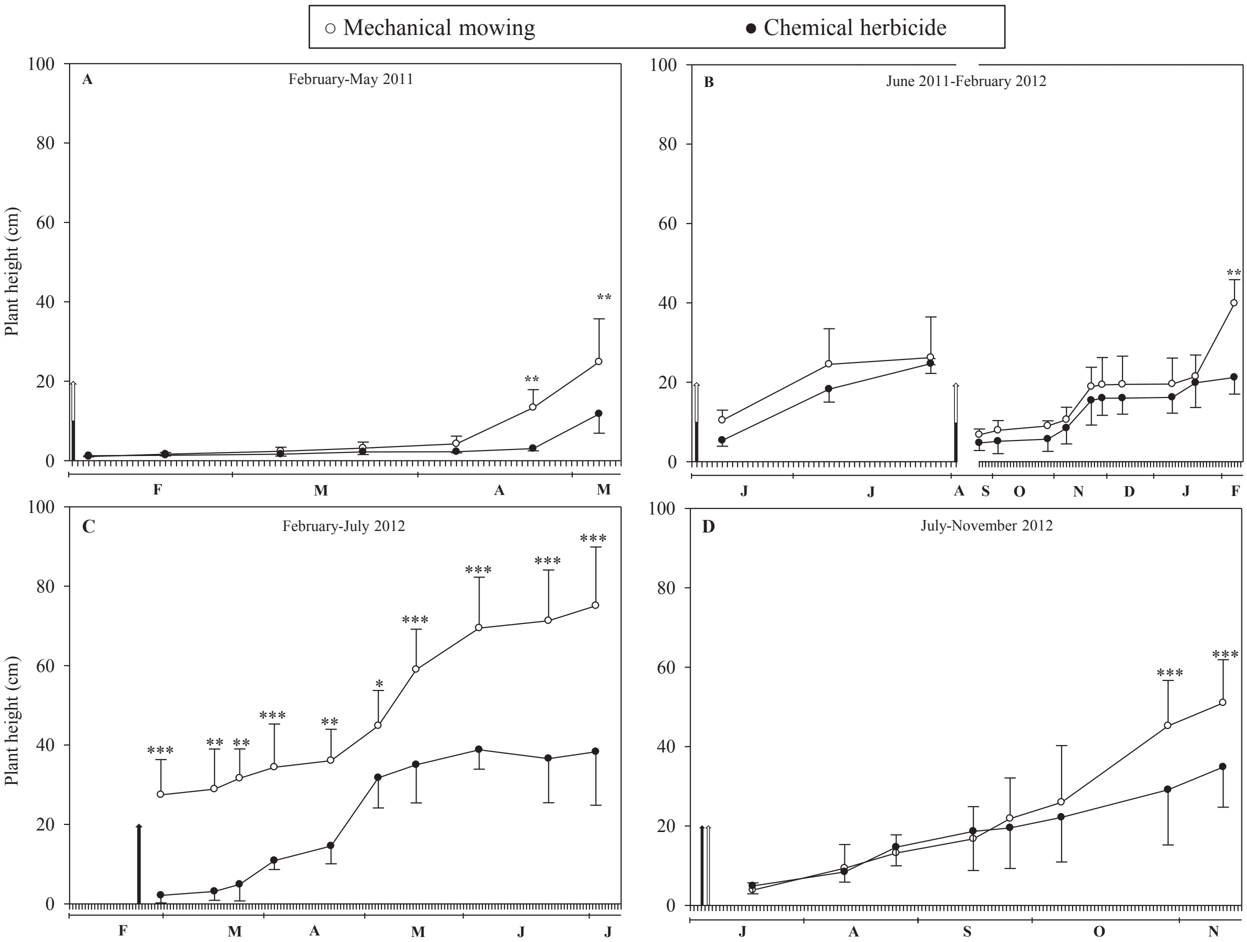
○ Mechanical mowing
● Chemical herbicide
A
February-May 2011
B
June 2011-February 2012
C
February-July 2012
D
July-November 2012
Plant height (cm)
Plant height (cm)
100
80
60
40
20
0
F
M
A
M
J
J
A
S
O
N
D
J
F
F
M
A
M
J
J
J
A
S
O
N
**
***
*

Fig. 1

Table

Table 1 From the top to the bottom: pH, electrical conductivity, average content of organic matter, assimilable P and total N in the plots to be mowed or subjected to treatment with the chemical herbicide at T_0 (20 January 2011) and in plots mowed or treated with the chemical at T_4 (7 November 2012) in the 0-10 cm and 10-30 cm vertical profiles. Mean values ± standard deviations are shown. In each column and row asterisks mark statistically significant differences (***: $P \leq 0.001$; **: $P \leq 0.01$; ns: $P > 0.05$, Student's-test)

		***T₀* Treatment**			***T₄* Treatment**		
	Depth (cm)	Mowing	Chemical	*P*	Mowing	Chemical	*P*
pH	0-10	7.8±0.15	7.9±0.05	ns	7.8±0.09	7.9±0.05	ns
	10-30	8.1±0.19	8.2±0.14	ns	8.1±0.12	8.1±0.13	ns
	P	ns	ns		ns	ns	
Electrical conductivity (µS cm^{-1})	0-10	96±25.5	95±13.7	ns	96±2.9	95±1.3	ns
	10-30	79±5.9	78±6.6	ns	77±4.6	79±10.6	ns
	P	ns	ns		ns	ns	
Organic matter (% in weight)	0-10	7.7±2.32	8.8±3.15	ns	4.9±1.12	5.8±2.49	**
	10-30	4.1±1.74	3.6±0.86	**	2.4±1.02	2.5±1.73	ns
	P	**	***		***	***	
Assimilable P (mg kg^{-1})	0-10	7.2±1.14	6.6±0.52	ns	6.9±1.08	7.2±4.63	ns
	10-30	3.5±0.59	4.0±0.40	*	2.5±0.14	2.7±0.69	ns
	P	***	**		**	***	
Total N (g kg^{-1})	0-10	2.5±0.51	2.8±0.69	ns	2.5±0.51	2.5±1.71	ns
	10-30	1.1±0.24	1.0±0.35	ns	0.9±0.14	0.9±0.15	ns
	P	***	***		**	***	

Table 2 Mean values (±standard deviation) of QBS-ar values at T_0 (20 January 2011), T_1 (16-18 May 2011), T_2 (31 January 2012), T_3 (3 July 2012) and T_4 (7 November 2012) for plots mowed or treated with the chemical herbicide. For each sampling time, none of the differences between treatments proved to be significant (P>0.05, Student's t-test)

Treatment	T_0	T_1	T_2	T_3	T_4
Mowed	35±15.6	33±19.0	57±15.2	60±4.1	20±17.8
Chemical	38±31.2	51±8.3	34±25.0	34±24.5	19±9.2

Table 3 Mean values (±standard deviation) of the biodiversity indices determined in the plots subjected to mechanical mowing or chemical treatment with the herbicide. Observations were performed at T_0 (20 January 2011), T_1 (16-18 May 2011), T_2 (31 January 2012), T_3 (3 July 2012) and T_4 (7 November 2012). For each sampling, none of the differences between treatments proved to be significant (*P*>0.05, Student's t-test)

			Shannon		
Treatment	T_0	T_1	T_2	T_3	T_4
Mowed	0.78±0.153	1.49±0.336	0.82±0.568	1.19±0.438	1.23±0.347
Chemical	0.66±0.298	1.30±0.527	1.06±0.639	0.74±0.290	1.13±0.150
			Simpson		
Treatment	T_0	T_1	T_2	T_3	T_4
Mowed	0.57±0.125	0.32±0.149	0.56±0.304	0.41±0.175	0.43±0.072
Chemical	0.66±0.167	0.35±0.189	0.48±0.279	0.59±0.189	0.36±0.081
			Equipartition		
Treatment	T_0	T_1	T_2	T_3	T_4
Mowed	0.34±0.091	0.80±0.181	0.55±0.436	0.72±0.263	0.71±0.198
Chemical	0.32±0.135	0.69±0.284	0.71±0.426	0.45±0.173	0.65±0.085

Table 4 Phytosociological table of the plant genus/species present in the plots to be mowed or subjected to treatments with the chemical herbicide, at T_0 (20 January 2011) and which were mowed or subjected to chemical treatments, at T_2 (2 February 2012) and T_4 (7 November 2012). Data represent the frequency of the genus/species. See text for details. Legend: - = not detected

	T₀								***T₂***								***T₄***							
	Mowing Plot #				Chemical Plot #				Mowing Plot #				Chemical Plot #				Mowing Plot #				Chemical Plot #			
Genus/Species	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Anagallis			II				II				III				I				I	I		I		IV
Arum				I	I			I		-				-				-				-		
Bellis			I				II			III				I					I					V
Bromus ramosus	I	V	III		I	V	I		I	III	IV	V	IV	III	V	IV	V	V	IV		III			
Capsella			I			-				-					I			-				-		
Cynodon		I			I					-				-				-				IV	III	
Daucus carota		-			I		I			-				-				-				-		
Equisetum		-				-				-			I			II		I	III			-		
Euphorbia				I	I	I				I			II	II			II	II		II	II			
Fumaria			I		I	I		II	I					-			I					-		
Galium	III	II		III	II	III	I		I	II	II	I	I	I	II	II	IV	II	II	II	I	II	I	III
Geranium		I	I			I	II	I		II	I			III	I	I			II	I		II		II
Lolium	V	III	V	V	V	III	V	V	IV	II		II	I	I	I			-				-		
Picris	I					-				-				-				-				-		
Plantago		I	I			-			I					-			II					II		
Poa		-				-			II				I	I			III				III		III	
Potentilla repens	II	III		III	V	I	I	I	III	IV			II		I	II	V	III		V			I	
Rubus		-				-					I				I			I	II			I		I
Rumex		I				-				-				-				-				-		
Senecium		-				-			I	II	I		III	III	II				III	III				
Silene		I		I	I					-				-				-				-		
Sonchus		II	I				I			-				-						I	I			
Trifolium		-				-				-				-			III				III		III	
Veronica	I	I			I		I		I				I	II	III	II		-			I	II	I	I

Table 5 Average (±standard deviation) content of selected trace elements (mg kg^{-1}, dry weight) in unwashed and washed samples of plants from plots to be mowed or subjected to treatments with the chemical herbicide, at T_0 (20 January 2011) and which were mowed or subjected to chemical treatments, at T_4 (7 November 2012). In each row, asterisks mark statistically significant differences (***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$; ns: $P > 0.05$, Student's-test). For each treatment, the temporal (T_4 vs T_0) increase/decrease ratio of the metals in unwashed and washed samples is reported

	Mowing			**Chemical**			**Mowing**			**Chemical**		
	T_0	**T_4**	**Δ (%)/*P***	**T_0**	**T_4**	**Δ (%)/*P***	**T_0**	**T_4**	**Δ (%)/*P***	**T_0**	**T_4**	**Δ (%)/*P***
	Unwashed			*Unwashed*			*Washed*			*Washed*		
Al	342	155	-55	520	151	-71	311	103	-67	296	107	-64
	±56.2	±67.4	(*)	±157.1	±74.3	(**)	±121.1	±55.5	(*)	±92.3	±56.7	(*)
As	2.5	0.5	-80	1.0	0.5	-50	0.9	0.4	-56	0.9	0.3	-67
	±1.37	±0.13	(**)	±0.24	±0.15	(*)	±0.54	±0.12	(ns)	±0.14	±0.14	(**)
Ba	84	25	-70	61	26	-57	46	19	-59	50	22	-56
	±38.2	±5.2	(***)	±19.4	±5.3	(**)	±15.8	±6.0	(**)	±8.0	±4.4	(***)
Cd	0.2	0.1	-50	0.1	0.1	0	0.1	0.1	0	0.1	0.1	0
	±0.08	±0.10	(**)	±0.04	±0.03	(ns)	±0.05	±0.02	(ns)	±0.043	±0.019	(ns)
Co	3.1	0.3	-90	2.1	0.4	-81	1.3	0.3	-77	1.2	0.2	-83
	±2.34	±0.16	(**)	±0.89	±0.26	(***)	±0.97	±0.11	(ns)	±0.32	±0.18	(***)
Cr	39	5	-87	30	6	-80	16	4	-75	15	4	-73
	±18.6	±1.9	(***)	±14.0	±2.4	(*)	±10.9	±1.8	(**)	±3.6	±1.8	(**)
Cs	0.3	0.1	-67	0.3	0.1	-67	0.2	0.1	-50	0.2	0.1	-50
	±0.16	±0.04	(*)	±0.09	±0.05	(*)	±0.08	±0.03	(***)	±0.03	±0.04	(**)
Cu	44	14	-68	27	14	-48	25	12	-52	20	11	-45
	±19.4	±3.0	(**)	±8.4	±4.2	(*)	±10.4	±2.3	(*)	±5.4	±3.5	(***)
Fe	4945	502	-90	2437	462	-81	1857	322	-83	1809	270	-85
	±3510.4	±282.8	(**)	±841.2	±298.1	(**)	±1224.3	±253.1	(*)	±514.7	±261.2	(***)
Mg	1938	1669	-14	1944	1617	-17	1492	1477	-1	1419	1298	-9
	±561.4	±126.2	(ns)	±337.3	±477.7	(ns)	±562.3	±191.0	(ns)	±351.3	±395.6	(ns)
Mn	257	55	-79	124	52	-58	95	43	-55	93	39	-58
	±114.3	±12.2	(**)	±47.7	±10.9	(*)	±52.1	±8.7	(ns)	±19.9	±8.7	(***)
Na	1563	1052	-33	1755	735	-58	1577	798	-49	1069	573	-46
	±234.5	±477.0	(ns)	±451.0	±398.5	(**)	±819.6	±367.2	(ns)	±315.2	±299.8	(ns)
Ni	34	5	-85	20	6	-70	16	4	-75	13	4	-69
	±24.0	±1.5	(***)	±7.5	±1.3	(***)	±11.4	±1.4	(*)	±2.1	±1.5	(***)
Pb	38	3	-92	18	3	-83	10	2	-80	11	2	-82
	±12.5	±1.0	(**)	±9.2	±1.7	(**)	±7.3	±0.9	(ns)	±2.0	±1.0	(***)
Sb	1.8	0.3	-83	1.1	0.4	-64	1.2	0.3	-75	0.9	0.3	-67
	±0.11	±0.19	(**)	±0.34	±0.25	(*)	±0.76	±0.20	(*)	±0.43	±0.20	(ns)
V	19	1.6	-92	7.2	1.4	-81	4.7	1.2	-74	4.5	1.0	-78

	±4.1	±0.66	(***)	±3.62	±0.61	(***)	±3.08	±0.56	(ns)	±1.05	±0.51	(**)
Zn	142 ±38.6	44 ±7.4	-69 (**)	84 ±27.7	49 ±11.0	-42 (*)	78 ±22.5	36 ±5.9	-54 (**)	66± 12.3	38± 7.3	-42 (**)

Table 6 Crustal enrichment factors for various elements in unwashed plants tissues sampled from plots to be mowed or subjected to treatments with the chemical herbicide at T_0 (20 January 2011) and which were mowed or subjected to chemical treatments, at T_4 (7 November 2012). None of the differences between treatments proved to be significant (P>0.05, Student's t-test). For each treatment, the temporal (T_4 vs T_0) increase/decrease ratio of enrichment factors in unwashed samples is reported

	Mowing		**Δ (%)/*P***	**Chemical**		**Δ (%)/*P***
	T_0	**T_4**		**T_0**	**T_4**	
Ba	20±4.1	25±7.2	+25 (ns)	15±3.6	18±0.6	+20 (ns)
Co	17±6.5	17±2.1	0 (ns)	23±4.8	20±5.1	-13 (ns)
Cr	64±18.6	76±9.3	+19 (ns)	95±14.7	95±37.7	0 (ns)
Fe	10±4.9	7±1.8	-30 (ns)	11±2.8	7±2.6	-36 (ns)
Mn	6±1.2	8±2.5	+33 (ns)	5±1.2	9±3.1	+80 (ns)
Ni	127±56.2	123±34.9	-3 (ns)	136±25.7	143±29.7	+5 (ns)
Pb	100±42.9	96±35.4	-4 (ns)	81±14.9	85±45.2	+5 (ns)
Sb	69±6.7	53±12.7	-23 (ns)	55±7.9	50±13.2	-9 (ns)
V	14±6.5	15±2.1	+7 (ns)	15±3.8	14±3.7	-7 (ns)

Table 7 Varimax rotation of principal factor pattern for elements in unwashed plant tissues sampled from plots to be mowed or subjected or subjected to treatments with the chemical herbicide at T_0 and at T_4 from plots subjected to mowing or to the treatment with the chemical herbicide. Only factor loadings with values over 0.40 are shown

			Parameter										*Eigenvalues*	*% total variance*	*Source type*
			Al	Fe	Co	Pb	Mn	V	Ni	Ba	Cr	Sb			
T_0	Mowing	***Factor 1***			0.74	0.98		0.51	0.54	0.71	0.72	0.68	*6.09*	*61.2*	**Vehicular traffic**
		Factor 2	0.81	0.59			0.54						*1.95*	*38.0*	**Crustal (soil)**
		Communalities	0.94	0.89	0.92	1.00	0.84	0.82	0.86	0.89	0.91	0.88		-	
	Chemical	***Factor 1***			0.89	0.90		0.91	0.84	0.83	0.79	0.88	*6.51*	*59.7*	**Vehicular traffic**
		Factor 2	0.75	0.62			0.71						*1.58*	*30.0*	**Crustal (soil)**
		Communalities	0.91	1.00	0.92	1.00	0.95	0.89	1.00	0.94	0.99	0.98		-	
T_4	Mowing	***Factor 1***				0.91		0.90	0.75	0.81	0.37	0.72	*8.01*	*81.2*	**Vehicular traffic**
		Factor 2	0.88	0.38			0.65						*1.15*	*17.1*	**Crustal (soil)**
		Communalities	0.95	0.99		0.79	1.00	1.00	0.68	0.98	0.71	0.89		-	
	Chemical	***Factor 1***			0.47	0.88			0.87	0.88	0.89	0.70	*6.49*	*78.8*	**Vehicular traffic**
		Factor 2	0.82	0.69			0.58						*1.44*	*13.9*	**Crustal (soil)**
		Communalities	0.94	0.89	0.92	1.00	0.84		0.86	0.89	0.91	0.88		-	