

# Hormesis and Paradoxical Effects of Drooping Birch (*Betula pendula* Roth) Parameters Under Motor Traffic Pollution

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## Abstract

Various plant indexes are used or recommended for bioindication. However, the nonmonotonic dose–response dependences (hormesis and paradoxical effects) of these indexes are insufficiently explored upon exposure to pollution. We studied the dependences of these *Betula pendula* indexes on the intensity of motor traffic pollution. Regression analysis did not reveal any dependence of chlorophyll and carotenoid content on traffic intensity (in 2008 and 2010–2013). Lipid peroxidation rate had different versions of paradoxical effects in 2008 and 2010 to 2012 and increased in comparison with control under an increase in pollution level in 2013. In 2010 to 2012, all dose–response dependences for total protein and thiol group content were biphasic and multiphasic paradoxical effects. In 2013, an increase in traffic intensity induced a linear reduction in protein content and an increase in thiol group level in comparison with the control. In most cases, the studied phenological indexes and seed production decreased monotonically in comparison with the control following an increase in traffic intensity. Only in 2010 and 2013, share of fallen leaves had hormesis and paradoxical effect accordingly. Fluctuating asymmetry had a paradoxical effect and hormesis in 2008 and 2012, accordingly, and increased in comparison with the control under an increase in the level of pollution in 2010 to 2011.

## Keywords

*Betula pendula* Roth, motor traffic pollution, plant physiological and morphological parameters, hormesis, paradoxical effects

## Introduction

At the present time, evidence has accumulated in toxicology that, apart from classical monotone dose–response dependences (S-shaped and exponential), nonmonotonic responses, which include hormesis (Cedergreen et al. 2007; Calabrese and Blain 2009) and paradoxical effects (Schatz 1999; Batyan et al. 2009; Smith et al. 2012), are also found rather often.

Hormesis is a biphasic dose–response phenomenon characterized by low-dose stimulation and high-dose inhibition (Calabrese 2008; Calabrese 2013). It is known that the manifestation of paradoxical effects consists of the following: as the dose or concentration of the toxic agent is reduced, its toxicity increases, and vice versa, such that with an increase in the dose, its effect is reduced (Schatz et al. 1964; Batyan et al. 2009).

Plants are nonmotile and cannot avoid the action of adverse environmental factors; therefore, various plant parameters are used in the bioindication for an estimation of environmental pollution. Photosynthetic pigment content (Rabe and Kreeb 1980; Carreras et al. 1996; Tripathi and Gautam 2007; Hassan et al. 2013), levels of total protein (Tripathi and Gautam 2007; Sing et al. 2013) and thiol groups (Ding et al. 1994), rate of

lipid peroxidation (Carreras et al. 1996; Rhoden et al. 2008; Hassan et al. 2013), leaf fluctuating asymmetry (Leung et al. 2000; Zakharov et al. 2000), phenological parameters (Sanz et al. 2011), and seed production (Savinov 1998) of various plant species are widely used or recommended for these purposes. However, the nonmonotonic dose–response dependences of these indexes have been insufficiently explored under different environmental pollution intensity in a wide range of values. This can result in the incorrect assessment of environmental quality by these indexes. Earlier, in experiments, we found that hormesis and paradoxical effects are observed more often than monotonic dose–response dependences under the influence of different chemical pollutants in a wide range of doses on the physiological, biochemical, and

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morphological plant parameters including rate of lipid peroxidation, chlorophyll and carotenoid content (Erofeeva 2014a), and leaf fluctuating asymmetry (Erofeeva 2012). It is quite possible that a similar phenomenon can be observed under the action of a complex of pollutants in urban areas if we consider a wide range of contamination levels.

We believe that it is necessary to study the dose–response dependences of bioindicative plant parameters for several years because the fluctuations of weather and pollution levels can change their pattern. Previously, in 2010 to 2011, we found different dose–response dependences of *Taraxacum officinale* seed reproduction parameters on motor traffic pollution intensity (Erofeeva 2014b). It was hypothesised that the significant differences in the studied dependencies were caused by changes in weather conditions because traffic intensity did not differ significantly between the 2 observation years (Erofeeva 2014b).

Road transport is a major source of air and soil pollution in most of the megacities of Russia, which has been caused by the rapid growth in the number of cars (Belkina, 2008). Drooping birch (*Betula pendula* Roth) often grows in roadside forest strips of cities in Russia. This species is a bioindicator, and many of its parameters are used for bioindication (Schebek et al. 1984; Kryazheva et al. 1996; Sameska-Cymerman et al. 2009). Therefore, this species of woody plant was selected for this study.

The aim of this study was to study nonmonotonic changes in *B. pendula* parameters such as photosynthetic pigment content, lipid peroxidation rate, phenological parameters, levels of total protein and thiol groups, seed production, and leaf fluctuating asymmetry upon exposure to motor traffic pollution in a wide range of values over several years of observation.

## Materials and Methods

### Plant Parameter Selection

For our study, we selected plant parameters that are widely used to estimate the level of environmental pollution and have a variable significance for the phenotypic adaptation of the plant. It is known that some products of lipid peroxidation, such as malondialdehyde (MDA), can regulate the function of proteins and gene activity and always take part in phenotypic plant adaptation to stress factors (Brand et al. 2004; Weber et al. 2004). Photosynthetic pigments do not have a similar biological activity and their content depends on the energy status and plastic plant metabolism (Mokronosov et al. 2006).

Protective plant peptides such as phytochelatins, metallothioneins, and glutathione have a lot of thiol groups. They are involved in the detoxification of heavy metals and are required for the functioning of the antioxidant system (Ha et al. 1999; Pompella et al. 2003; Felizola et al. 2014). Total protein content depends on the synthesis of protective proteins such as antioxidant enzymes, stress, and signaling proteins (Srivastava et al. 2005).

Fluctuating asymmetry is widely used to estimate the developmental stability of bilateral morphological structures of the plant leaf. It is understood as random insignificant deviations from the symmetrical state (Zakharov et al. 2001) owing to the stochastic nature of molecular processes that provide the expression of genes (ontogenetic noise; Leamy and Klingenberg 2005).

Phenological indexes and seed reproduction parameters depend on plant capacity to maintain phenological rhythms (Honour et al. 2009) and to carry out sexual reproduction under environmental stress (Levina 1981).

### Study Area and Study Plots

Our research was carried out in 2008 and in 2010 to 2013. We studied parameters in middle-aged generative trees of *B. pendula*. The trees grew in 9 to 10 model areas (plots) of tree stands planted along roadsides in the upland part of the city of Nizhni Novgorod, Russia. Motor traffic is a major source of pollution in this part of the city. All plots were characterized by similar soil conditions (light gray forest soils with anthropogenically mixed upper horizons) and a normal moistening regime. Their location was chosen so that traffic intensity varied within a wide range, with the minimum and maximum values differing by a factor of several tens. A conditionally clean area near the village of Kiselikha, 20 km north of Nizhni Novgorod, was chosen as the control plot. The control plot was located far from highways and other pollution sources. Leaf samples for physiological and morphological analyses were collected from shortened shoots of 10 trees of every plot on the side of the crown facing the road, at a height of 2 to 3 m.

### Estimation of Motor Traffic Pollution

Motor traffic pollution was estimated by the traffic intensity (vehicles/h). The traffic intensity was the median of vehicles per hour, counted 3 times on a weekday: in the morning (from 8 until 10), in the afternoon (from 12 until 15), and in the evening (from 17 until 19; Ruzskiy et al. 2008). We previously demonstrated that the traffic intensity was correlated with the content of the main pollutants (oxides of sulfur, nitrogen, carbon, benzene, kerosene, benzo[a]pyrene, and formaldehyde) in the air along highways in Nizhni Novgorod ( $r = .8-.9$ ;  $P < .05$ ).

### Photosynthetic Pigments

The photosynthetic pigment content was estimated in pooled samples of 30 to 35 leaves from each of the 10 trees per plot (10 trees/plot;  $n = 10$ ) in 2008 and 2010 to 2013.

Fresh leaves (0.2 g) were cut into pieces and homogenized in 80% acetone in the presence of quartz and calcium carbonate. The homogenate was filtered and then diluted to 25 mL with 80% acetone. The absorbance of the mixture was measured at 470, 646, and 663 nm using a UV–visible spectrophotometer (SF 2000; OKB Spectrum, Russia). The concentrations

of chlorophylls and carotenoids were calculated according to the formulae of Lichtenthaler (1987).

### Lipid Peroxidation

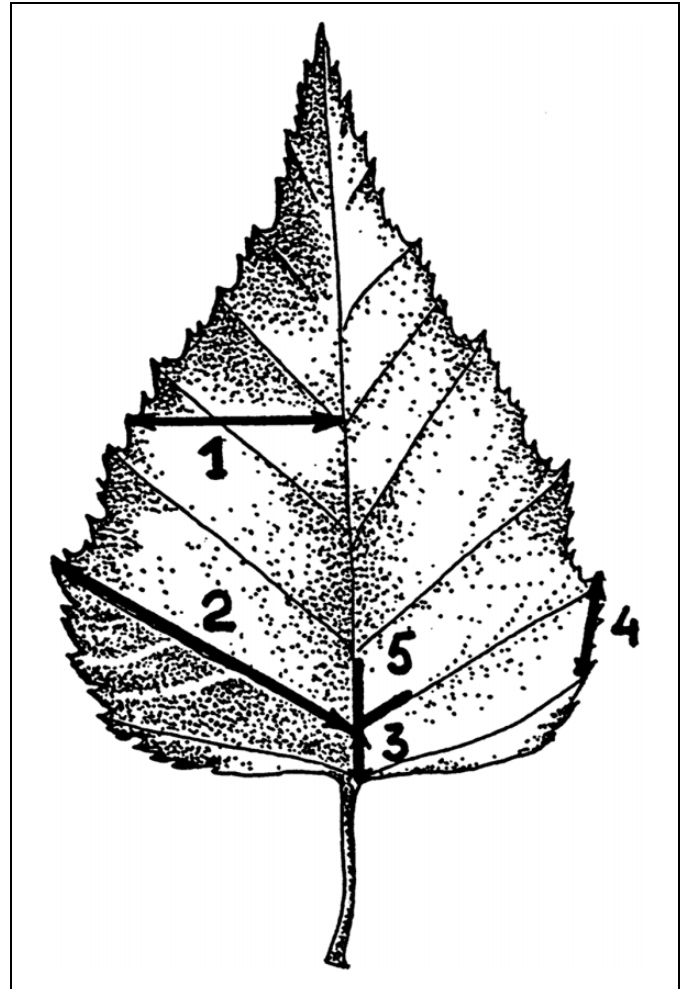
Lipid peroxidation rate was estimated in pooled samples of 30 to 35 leaves from each of the 10 trees per plot (10 trees/plot;  $n = 10$ ) in 2008 and 2010 to 2013. The level of lipid peroxidation was determined in terms of MDA concentration according to the method of Heath and Packer (1968) with the modifications of Kamyshnikov (2002). Approximately 0.2 g of fresh leaves was homogenized in 2 mL of 3 mmol/L EDTA. Then, 2 mL of 20% trichloroacetic acid and 2 mL of 0.75% 2-thiobarbituric acid were added to 2 mL of the homogenate. The mixture was incubated at 96°C for 30 minutes and then transferred into an ice bath to stop the reaction. The tubes were centrifuged at 10 000g for 15 minutes, and the absorbance of the resulting supernatant was measured at 532 nm using spectrophotometer SF 2000. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using the extinction coefficient of 155 mmol/L<sup>-1</sup> cm<sup>-1</sup>. The concentration of MDA was expressed as nmol g<sup>-1</sup> FW (fresh weight of leaf).

### Thiol Groups and Total Protein

Thiol group level and total protein content were estimated in pooled samples of 30 to 35 leaves from each of the 10 trees/plot ( $n = 10$ ) in 2010 to 2013. The level of thiol groups was determined according to the method of Ellman (1959), with insignificant modifications. Approximately 0.3 g of fresh leaves was homogenized in 3 mL of 0.1 mol/L Tris-HCl (pH = 7.8-8) buffer. The tubes were centrifuged at 10 000g for 10 minutes. Then, 2 mL of 0.1 mol/L Tris-HCl (pH = 7.8-8) buffer and 0.3 mL of sodium dodecyl sulfate were added to 0.3 mL of the supernatant. The absorbance of the resulting solution was measured at 412 nm using spectrophotometer SF 2000. Then 0.2 mL of 50 μmol/L Ellman reagent, 5,5'-dithiobis-(2-nitrobenzoic acid), was added into the tubes, and the absorbance of the resulting solution was measured at 412 nm after tube incubation at 37°C for 60 minutes. The difference in absorption of the solution was found after incubation and before the addition of Ellman reagent. Thiol group concentration was calculated using the extinction coefficient of 13.6 mmol/L<sup>-1</sup> cm<sup>-1</sup>. The concentration of thiol groups was expressed as μmol/L g<sup>-1</sup> total protein in FW of leaf. Total protein content was found according to the method of Bradford (1976). Bovine serum albumin was used as a standard.

### Phenological Indexes

We estimated phenological indexes such as share of blossom buds (in May or in April) and share of fallen leaves (in September or in October) in 10 trees of each plot in 2010 to 2013. Earlier, we supposed using these parameters for woody plants to estimate the emergence rate from winter

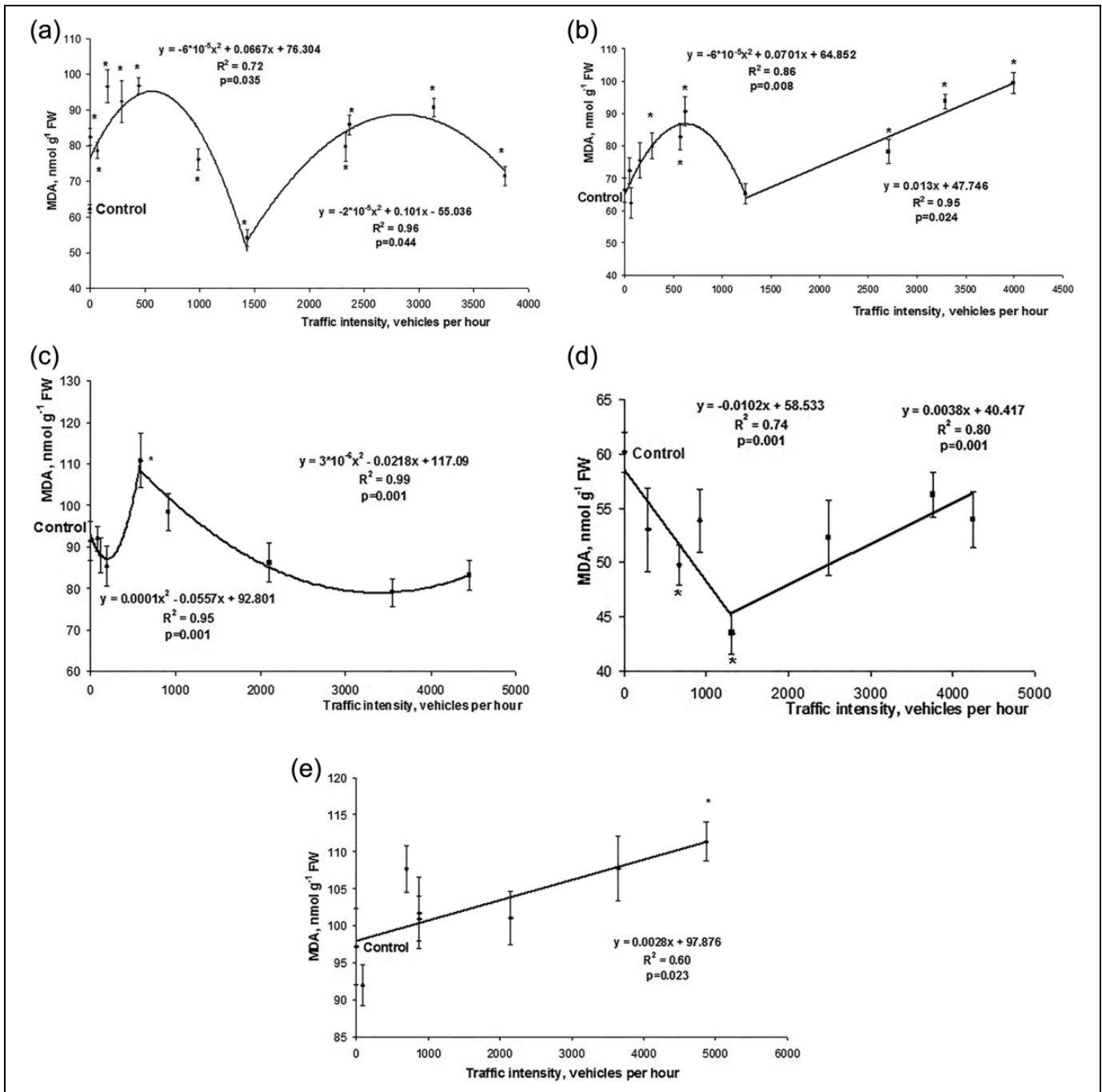


**Figure 1.** Measurements of *Betula pendula* leaf: (1) 1/2 of the width of the leaf in the area of 1/2 of the length of the central vein; (2) length of the second from the bottom vein of the second order; (3) distance between the bases of the first and the second from the bottom veins of the second order; (4) distance between the ends of the first and the second from the bottom veins of the second order; and (5) angle between the central vein and the second from the bottom vein of the second order.

dormancy in spring (share of blossom buds) and vegetation period termination (share of fallen leaves) in autumn (Erofeeva 2011).

To estimate the share of blossom buds, we studied the state of 100 buds in the middle and lower parts of the crown of each tree from the motorway (20 buds in 5 different parts of the crown of the tree). A bud was considered a blossom bud if the leaf was outside it. Then, the share of blossom buds in the pooled sample of buds for all 10 trees of the plot was calculated (10 trees × 100 buds;  $n = 1000$ ).

To estimate the share of fallen leaves, we studied the state of 100 shoot nodes in the middle and lower parts of the crown of each tree from the motorway (20 shoot nodes in 5 different parts of the crown of the tree). Each node of shoot has only 1 leaf, therefore, using the number of nodes without leaves we determined the number of fallen leaves. Then, the share of



**Figure 2.** Dependence of lipid peroxidation rate on motor traffic intensity in the *Betula pendula* leaf in 2008 (a), 2010 (b), 2011 (c), 2012 (d), and 2013 (e). FW, fresh weight of leaf.

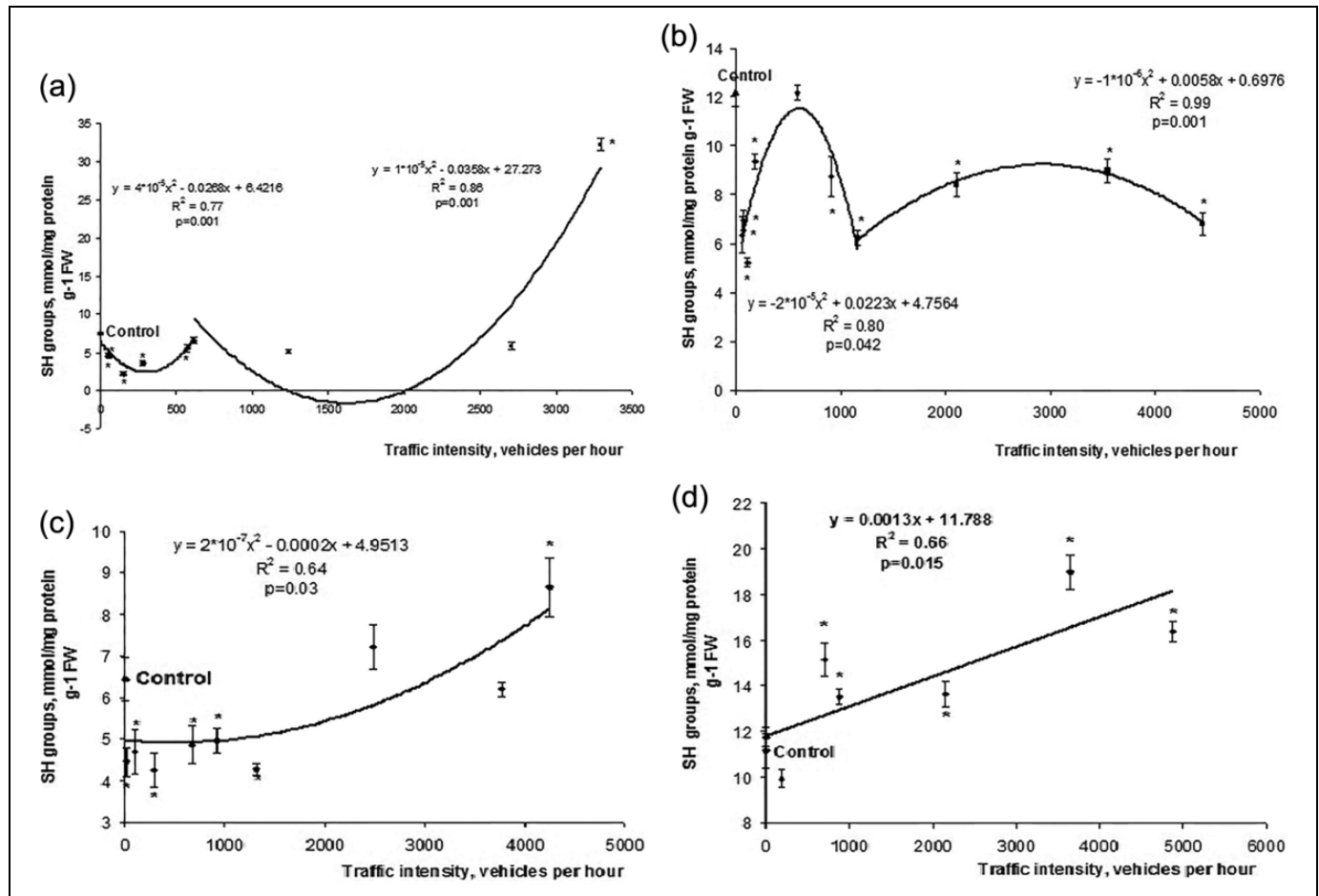
\* indicates significant differences between treatments and control at  $P < .05$ .

fallen leaves in the pooled sample of nodes for all 10 trees of the plot was calculated (10 trees  $\times$  100 nodes;  $n = 1000$ ).

### Seed Production

To estimate *B. pendula* seed reproduction, we used a relative seed production in 2010 to 2013, which was previously proposed by us as a new index of tree seed reproduction (Erofeeva

2014c). The relative seed production of a coenopopulation is calculated as a percentage of the maximum seed production of a coenopopulation when there are seeds on all plant organs that can have generative buds (Erofeeva 2014c). We estimated seed production in 10 trees of each plot when seeds started to mature but still did not fall (the third decade of June). *Betula pendula* has seeds only on short shoots, therefore, the number of shoots with and without seeds was estimated. We studied 100 short



**Figure 3.** Dependence of thiol group content on motor traffic intensity in the *Betula pendula* leaf in 2010 (a), 2011 (b), 2012 (c), and 2013 (d). \* indicates significant differences between treatments and control at  $P < .05$ . FW, fresh weight of leaf.

shoots in the middle and lower part of the crown of each tree from the motorway (20 short shoots in 5 different parts of the crown of the tree). Then, the percentage of short shoots with seeds in the pooled sample of short shoots for all 10 trees of the plot was calculated (10 trees  $\times$  100 short shoots;  $n = 1000$ ).

### Fluctuating Asymmetry

To estimate fluctuating asymmetry, 10 leaves from each of the 10 trees per plot (10 leaves  $\times$  10 trees;  $n = 100$ ) were collected and analyzed for the standard set of 5 morphological characters (Zakharov et al. 2000; Figure 1) in 2008 and 2010 to 2012. On this basis, the integrated fluctuating asymmetry index was calculated using the normalized difference algorithm (Zakharov et al. 2000):

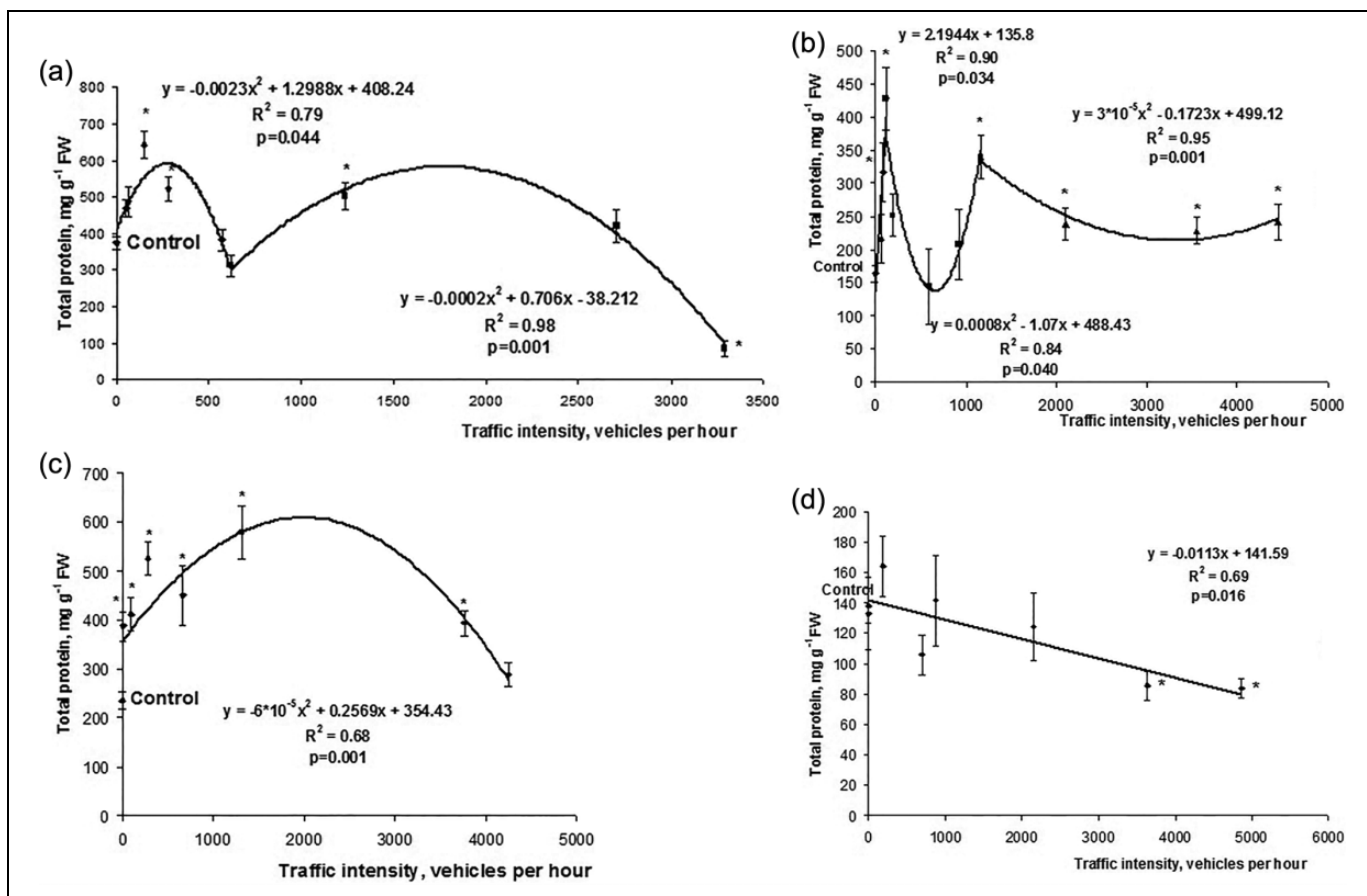
$$\bar{A} = \frac{1}{m \cdot n} \sum_{i=1}^m \sum_{j=1}^n \frac{(L_{ij} - R_{ij})}{(L_{ij} + R_{ij})},$$

where  $L_{ij}$  and  $R_{ij}$  are the values of the  $j$ th character on the right and left sides of the  $i$ th leaf, respectively;  $m$ —number of studied morphological characters;  $n$ —sample size of leaves. The integrated

fluctuating asymmetry index was used to assess the quality of the environment with a score scale (Zakharov et al. 2000).

### Statistical Analysis

Statistical analyses were executed using the programs Statistica 8.0. and Primer of Biostatistics 4.03. Parametric criteria were used since the Shapiro-Wilk criterion showed that the sampling distribution in all treatments and controls did not differ from the Gaussian distribution. One-way analysis of variance and parametric Newman-Keuls tests were used for multiple comparisons of studied quantitative parameters. Chi-square test with the Bonferroni correction was used for multiple comparisons of the shares. Regression analysis was used to evaluate the dependence of the studied parameters on traffic intensity. One or two points of some dose–response dependences were outside the 95% confidence interval of the values, therefore, they were excluded from regression analysis. Exclusion of such points from regression analysis is an accepted procedure in statistics (Glantz 2005). Sampling means with standard errors were used for graphical data presentation. The least significant difference was used for multiple comparison at the  $P < .05$  level between treatment and control means.



**Figure 4.** Dependence of total protein content on motor traffic intensity in the *Betula pendula* leaf in 2010 (a), 2011 (b), 2012 (c), and 2013 (d). \* indicates significant differences between treatments and control at  $P < .05$ . FW, fresh weight of leaf.

## Results

### Photosynthetic Pigments

Regression analysis did not reveal any dependence of chlorophyll and carotenoid content in leaf on motor traffic intensity in 2008 and 2010 to 2013. Therefore, these data were not presented in the figures. Apparently, the adaptation of *B. pendula* to pollution required considerable energy expenditure. Therefore, the maintenance of photosynthesis (including the content of photosynthetic pigments) as an energy source was necessary for the survival of *B. pendula* in extreme conditions.

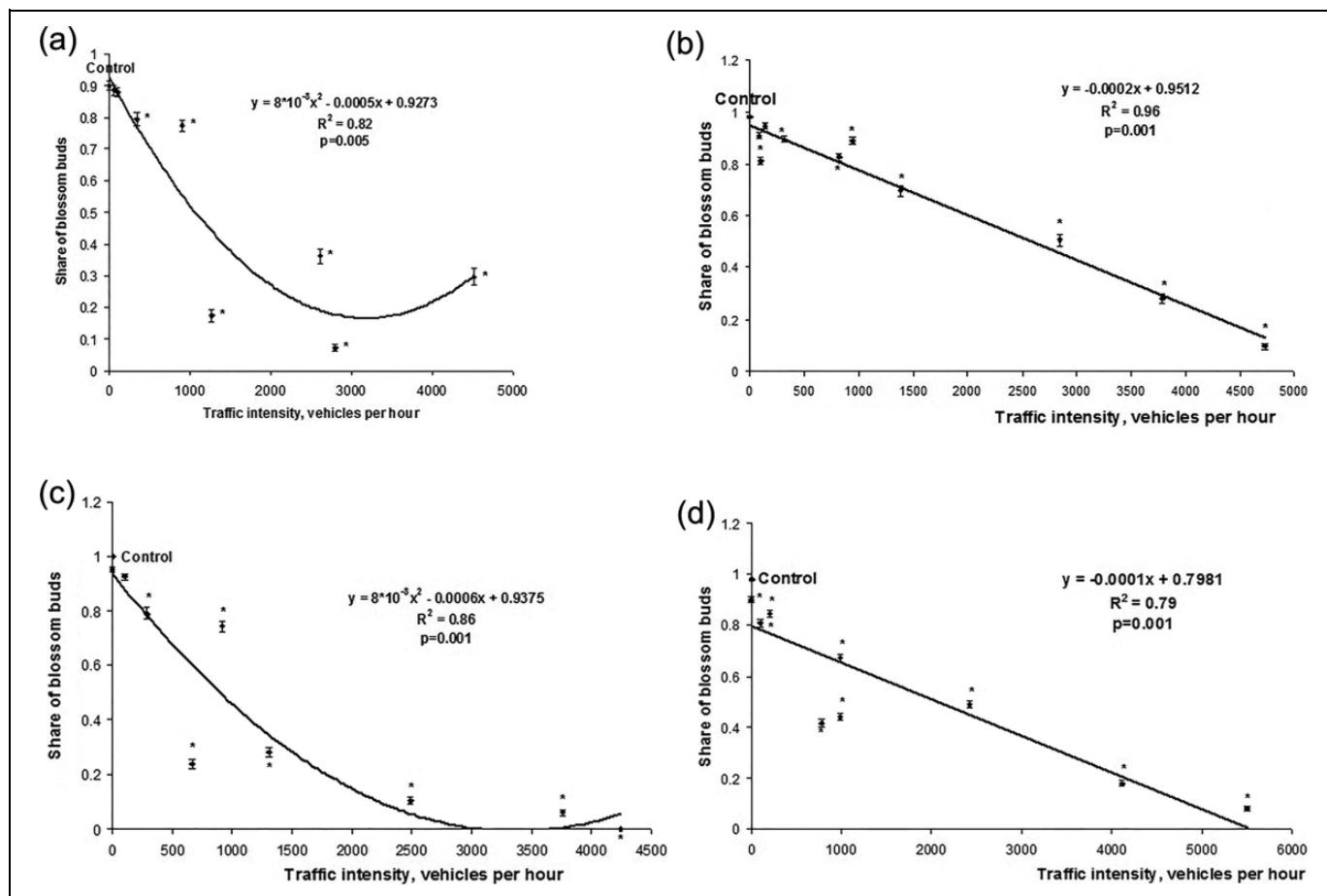
### Lipid Peroxidation

Regression analysis revealed a nonmonotonic dependence of lipid peroxidation rate on traffic load in all years of observation (2008 and 2010–2012) except 2013. These dependences were paradoxical effects (Figure 2a–d). In 2013, a linear increase was found in lipid peroxidation rate in *B. pendula* leaf (Figure 2e). There was a statistically significant value of this parameter in comparison with the control only under the highest load (Figure 2e). According to a commonly held view, an environmental stress increases the lipid peroxidation rate (Ayala et al. 2014; Polesskaya 2007); however, we found that the more complex

nonmonotonic dose–response dependences were more widespread. In 2011 to 2012, biphasic paradoxical effects were observed (Figure 2c–d). In the first phase, an increase (or decrease) in the studied parameter in comparison with the control was revealed and then lipid peroxidation rate was normalized. In 2008 and 2010, dose–response dependences were multiphasic. In 2008, the dose–response dependence consisted of 2 oscillations (Figure 2a). In 2010, dose–response dependence included an oscillation and a linear increase in the parameter in comparison with the control under the high traffic intensity (Figure 2b).

### Thiol Groups and Total Protein

Regression analysis revealed dependences of thiol group level and total protein content on traffic load in all of the years of observation (2010–2013). A negative correlation between thiol group level and total protein content was found in 2010 to 2013 ( $r = -0.72$  to  $-0.94$ ;  $P < .05$ ). In 2013, an increase in traffic intensity induced a linear reduction in protein content and an increase in thiol group level in comparison with the control (Figures 3d and 4d). In 2010 to 2012, all dose–response dependences were biphasic or multiphase paradoxical effects (Figures 3a–c and 4a–c). It should be noted that in most cases,



**Figure 5.** Dependence of share of *Betula pendula* blossom buds on motor traffic intensity in 2010 (a), 2011 (b), 2012 (c), and 2013 (d). \* indicates significant differences between treatments and control at  $P < .05$ .

the low and medium traffic intensity caused a decrease in thiol group level and an increase in the protein content. On the contrary, high traffic led to a decrease in protein level and an increase in group content as a rule (Figures 3 and 4). Apparently, moderate levels of contamination induced the synthesis of a number of protective and regulatory proteins. At high pollution intensity, the total protein content could be reduced due to the energy deficit on the synthesis of these macromolecules as well as a result of their oxidation. Therefore, at high traffic pollution, the synthesis of protecting peptides containing thiol groups (such as phytochelatin, metallothioneins and glutathione) was increased because it requires less energy expenditure.

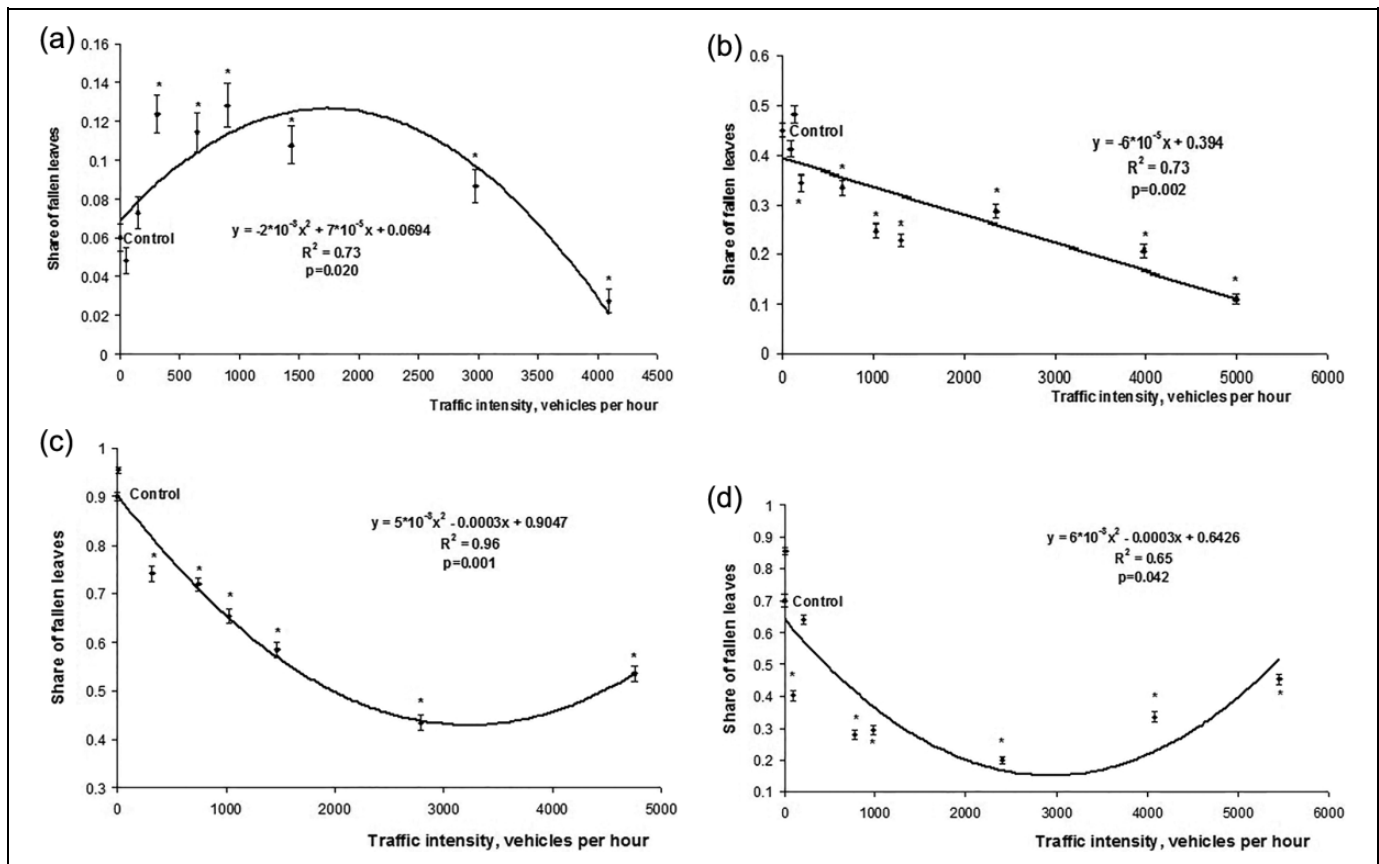
### Phenological Indexes

Dependencies of share of blossom buds and share of fallen leaves on traffic intensity were most adequately described by line equations or quadratic polynomial equations and had a distinct monotonic pattern in most cases. The increase in traffic intensity caused a significant decrease in the studied

parameters of *B. pendula* in comparison with the control in most cases (Figures 5a-d and 6a-d). This fact indicated that *B. pendula* started and finished vegetation later in the polluted plots in comparison with the control. Our data are consistent with the results of other authors who showed a decrease in emergence rate from winter dormancy for other tree species upon exposure to environmental pollution (Vonsury and Fluckiger 1991). In 2010 and 2013, the dose-response dependences of the fallen leaves were hormesis and paradoxical effect, accordingly (Figure 6a and d).

### Seed Production

The dependence of percentage of short shoots with seeds on traffic intensity was most adequately described by a quadratic polynomial equation (in 2010–2011 and 2013) or line equation (in 2012) and had a distinct monotonic pattern (Figure 7a-d). An increase in traffic intensity caused a significant decrease in the studied parameter of *B. pendula* in comparison with control (up to absolute absence of seeds on trees; Figure 7a-d). Our data are consistent with the results of other authors who found a



**Figure 6.** Dependence of share of *Betula pendula* fallen leaves on motor traffic intensity in 2010 (a), 2011 (b), 2012 (c), and 2013 (d). \* indicates significant differences between treatments and control at  $P < .05$ .

reduction in seed reproduction in plant under environmental pollution (Zvereva et al 2010).

### Fluctuating Asymmetry

In 2008 and 2010 to 2011, monotonic dependences of fluctuating asymmetry index on traffic intensity were found, which were described by a quadratic polynomial or linear equations. An increase in traffic induced an increase in fluctuating asymmetry index and environmental quality scores (Figure 8a-c).

In 2008, fluctuating asymmetry index was very high in control plot; therefore, it was outside the 95% confidence interval of the values. It is advised to exclude such values in regression analysis. Therefore, we presented a regression equation with a control point and without. In 2008, environmental state was critical in control plot (5 scores; Figure 8a). Although this dependence is linear, it can be attributed to paradoxical effects because at traffic intensity  $<1500$  vehicles/h a decrease in the studied parameter in comparison with the control was revealed, and at high traffic ( $>1500$  vehicles/h) fluctuating asymmetry index did not differ from the control ( $P > .05$ ).

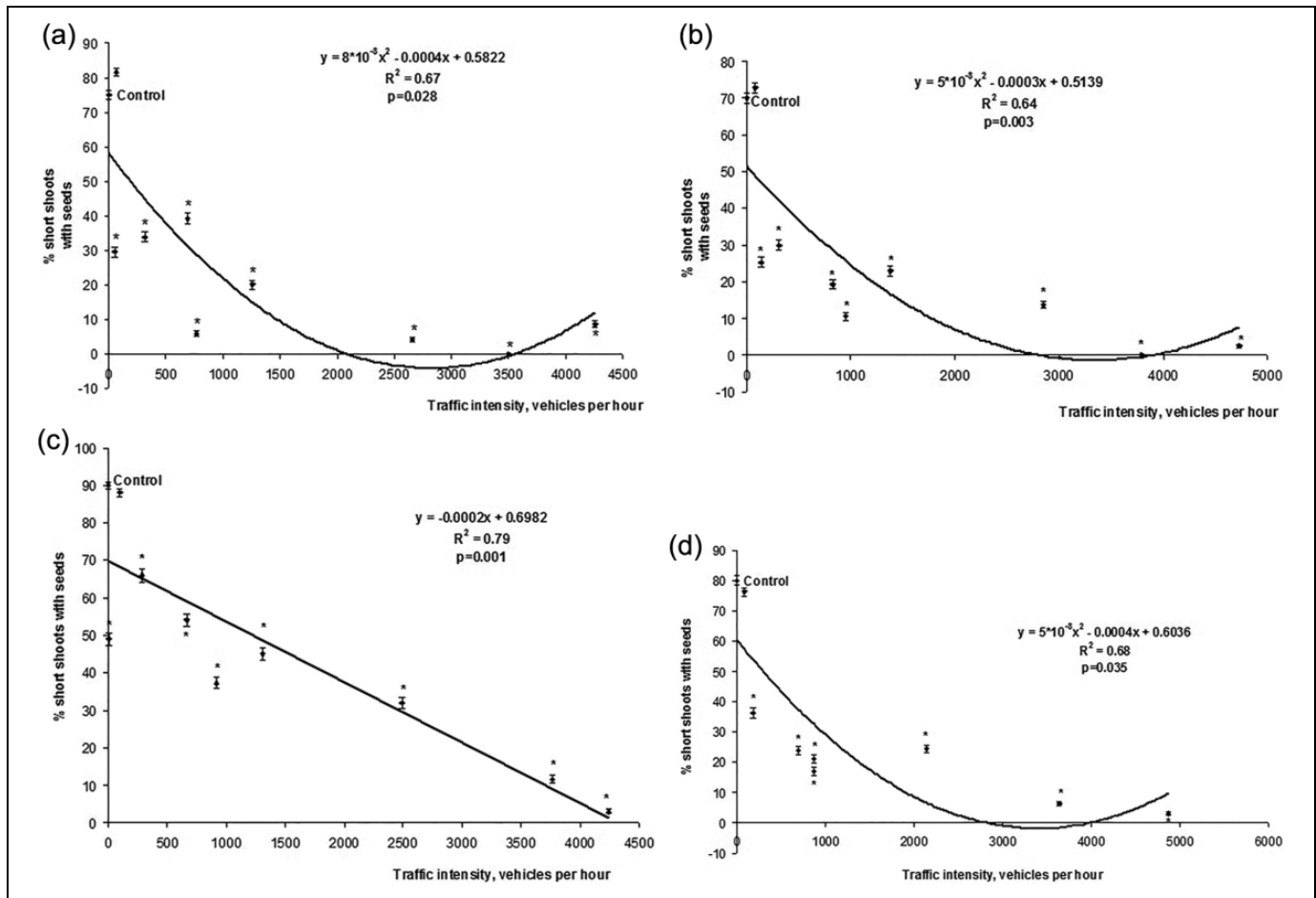
In 2012, the dependence of fluctuating asymmetry index on traffic intensity was biphasic (Figure 8d). Although statistically significant differences with control for fluctuating asymmetry

index were only identified in the trees on the plot with the highest traffic load, scores of environmental quality differed in the studied plots. At the first phase of dependence, an increase in traffic intensity (to 915 vehicles/h) induced a decrease in scores to 2 (insignificant deviation from norm), whereas it was estimated by 3 scores (middle deviation from norm) in the control and 4 scores (significant deviation from norm) in the plot with minimum load. At the second phase, maximum transport load (4245 vehicles/h) increased scores to 5 (critical state; Figure 8d).

### Discussion

Thus, the *B. pendula* parameters that are directly involved in the plant organism phenotypic adaptation (lipid peroxidation rate, thiol group level, and total protein content) had predominantly nonmonotonic dose-response dependences (Figures 2–4). It is known that some products of lipid peroxidation, such as MDA, can regulate the function of proteins and gene activity and always take part in phenotypic adaptation to stress factors in plants (Brand et al. 2004; Weber et al. 2004). Thiol group level depends on the content of protective plant peptides such as phytochelatins, metallothioneins, and glutathione. They are involved in the detoxification of heavy metals and are required





**Figure 7.** Dependence of *Betula pendula* seed production on motor traffic intensity in 2010 (a), 2011 (b), 2012 (c), and 2013 (d). \* indicates significant differences between treatments and control at  $P < .05$ .

for functioning of the antioxidant system (Ha et al. 1999; Pompella et al. 2003; Felizola et al. 2014). Total protein content depends on the synthesis of protective proteins such as antioxidant enzymes and stress and signaling proteins (Srivastava et al. 2005).

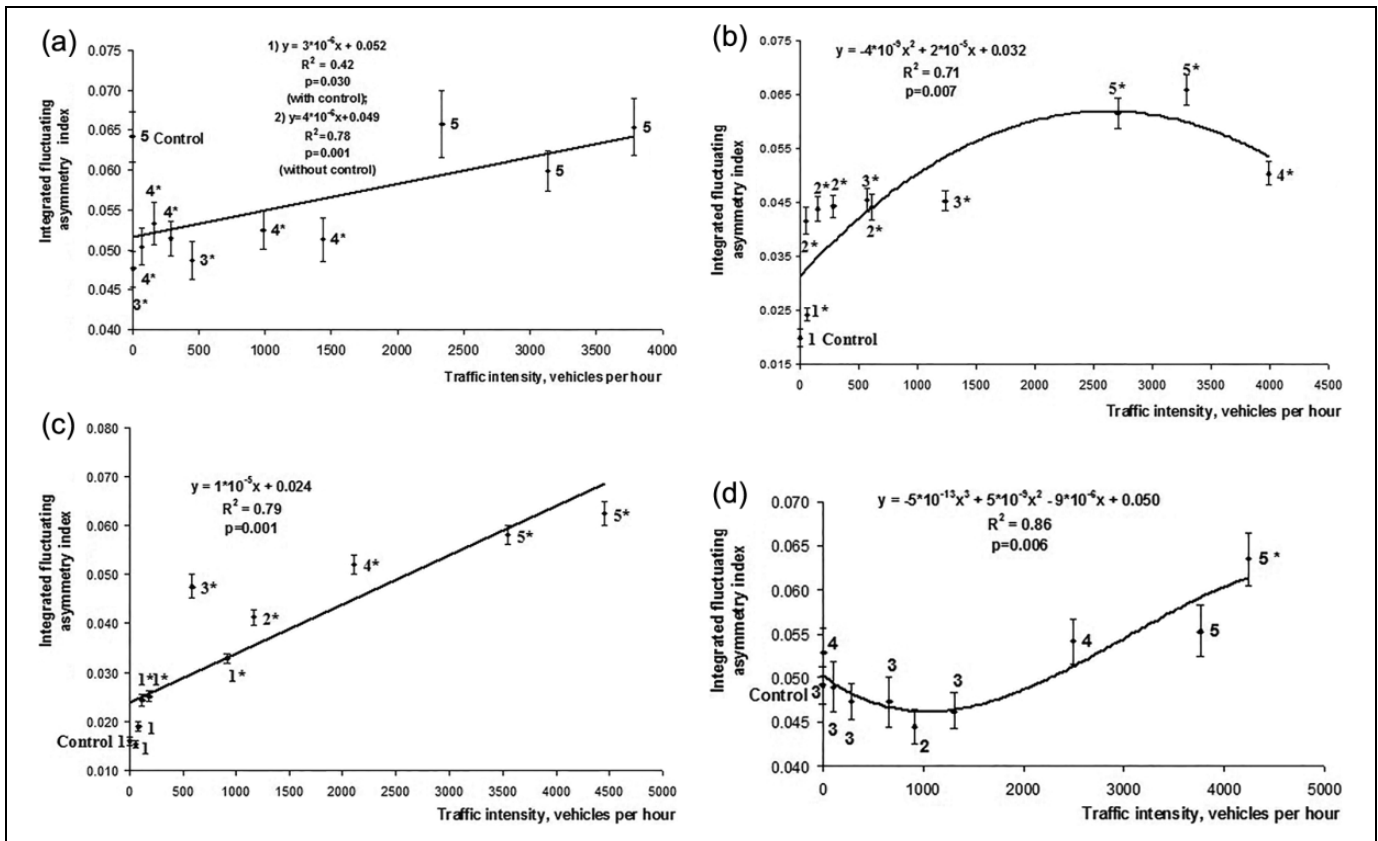
On the contrary, the *B. pendula* parameters that are less important for the tree existence under environmental stress (phenological indexes, seed production, and leaf fluctuating asymmetry) monotonically worsened in most cases under an increase in traffic pollution (Figures 5–8). There was a reduction in seed production, an increase in the disturbance of phenological indexes, and an increase in leaf fluctuating asymmetry caused by a reduction in the developmental stability of leaf morphological structures. Apparently, the adaptation of *B. pendula* to motor traffic pollution requires considerable energy expenditure. Therefore, *B. pendula* “saves recourses” on seed production, and the maintenance of leaf developmental stability and phenological rhythms.

Our studies have shown that the studied *B. pendula* parameters can have different types of dose–response dependences in the same year of observation. In most cases, this phenomenon was found to include lipid peroxidation rate and leaf

fluctuating asymmetry (Figures 2 and 8), both of which are considered indicators of environmental stress level (Zakharov et al. 2001; Leamy and Klingenberg 2005; Poleskaya 2007). Hence, the assessment of the environmental stress level with the use of these indicators can differ.

We also found differences between patterns of dose–response dependences of the same studied parameters in different years of observation. For example, the lipid peroxidation rate had different versions of paradoxical effects in 2008 and 2010 to 2012 (Figure 2a–d) and increased linearly under an increase in the level of pollution in 2013 (Figure 2e). This may be caused by fluctuations of traffic intensity and weather conditions. The influence of weather fluctuations on the pattern of dose–response dependences was confirmed by the statistically significant differences between the studied parameters of trees in the control plot in different years of observation ( $P < .05$ ).

Our studies have shown that it is necessary to study dose–response dependencies for the correct choice of bioindicative plant parameters over several years of observation. Besides, one should explore a wide range of pollution intensity. Plant parameters that are not involved directly in the organism phenotypic adaptation to anthropogenic load are more suitable for



**Figure 8.** Dependence of *Betula pendula* leaf fluctuating asymmetry on motor traffic intensity in 2008 (a), 2010 (b), 2011 (c), and 2012 (d). \* indicates significant differences between treatments and control at  $P < .05$ . Arabic numerals are the scores of environmental quality calculated by the score scale for the integrated fluctuating asymmetry index (FAI; Zakharov et al. 2000): 1 (FAI < 0.040)—a norm, 2 (FAI = 0.040-0.044)—an insignificant deviation from norm, 3 (FAI = 0.045-0.049)—a middle deviation from norm, 4 (FAI = 0.050-0.054)—a significant deviation from norm, and 5 (FAI > 0.054)—a critical state.

bioindication because they can worsen monotonically under an increase in pollution level in a wide range of values. Only a share of blossom buds and seed production of all the studied *B. pendula* parameters were suitable for the evaluation of motor traffic pollution intensity because they had monotonic dose-response dependences for all of the years of observation (Figures 5a-d and 7a-d).

Hormesis could be part of some multiphasic dose-response dependences (ie, part of the paradoxical effects). For example, some parts of the multiphasic dose-response dependences (ie, paradoxical effects) of lipid peroxidation rate and total protein content in 2008 (1500-4000 vehicles/h; Figure 2a) and in 2010 (500-3500 vehicles/h; Figure 4a), accordingly, were a hormesis. In the first phase of hormesis, traffic pollution induced an antioxidant effect and increase in total protein content, while in the second phase of hormesis, a prooxidant effect and decrease in protein level were seen (Figures 2a and 4a). Previously, we have found a similar phenomenon under the action of various pollutants on parameters of wheat seedlings in the experiment (Erofeeva 2014a). Thus, paradoxical effects and hormesis, apparently, are interrelated phenomena as they can be parts of the same complex oscillatory dependence.

At present, the causes of a nonmonotonic response in living organisms are insufficiently explored. At the same time, it was shown that at different phases of nonmonotonic dose-response dependence, an agent can act on different receptor types (subtypes) or different cell signaling pathways that induce the nonmonotonic pattern of the dependence (Calabrese 2013). Previously, to explain this phenomenon, we proposed a hypothesis (Erofeeva et al. 2011; Erofeeva 2014a) that was based on the concept of the gradual involvement of different adaptive mechanisms in the process of phenotypic adaptation to a factor and the existence of several regimes (levels) of functioning in living organisms (Veselova et al. 1993; Garkavi et al. 1998).

We can draw the following conclusions based on this study:

1. Regression analysis did not reveal any dependence of chlorophyll and carotenoid content in *B. pendula* leaf on motor traffic intensity for any of the years of observation.
2. Lipid peroxidation rate had different versions of paradoxical effects in 2008 and in 2010 to 2012 and increased linearly under an increase in the level of pollution in 2013 in comparison with the control.

3. In 2010 to 2012, all dose–response dependences for total protein and thiol group content were biphasic or multiphase paradoxical effects. In 2013, the increase in traffic intensity induced a linear reduction in protein content and an increase in thiol group level in comparison with the control.
4. In most cases, the investigated phenological indexes (share of blossom buds and fallen leaves) had monotone dose–response dependences and decreased in comparison with the control under an increase in traffic intensity. Only in 2010 and 2013, share of fallen leaves had hormesis and paradoxical effect accordingly.
5. Seed production decreased monotonically in comparison with the control under an increase in traffic intensity for all of the years of observation.
6. Leaf fluctuating asymmetry had a paradoxical effect and hormesis in 2008 and 2012, accordingly, and increased monotonically in comparison with the control under an increase in the level of pollution in 2010 to 2011.

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