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Epiphytic lichens associated with different traffic intensities along the highway E4

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Photo: Chiho Okuyama

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Sammanfattning

En lav är en symbiotisk enhet som består av en svamp och en grönalg eller en cyanobakterie. Lavar är känsliga för luftföroreningar och sedan 1800-talet har de använts för att mäta luftföroreningar. Trots att lavar är användbara indikatorer, är det fortfarande oklart hur snabbt de kan reagera på förändringen av luftkvaliteten.

Vägtrafikens luftföroreningar skapar bland annat miljöproblem som påverkar såväl människor som djur och växter. Det finns olika föroreningsämnen som släpps ut ur förbranningsmotorer, t.ex. svävedioxid (SO_2), kväveoxider (NO_x), ozon (O_3), polycykliska aromatiska kolväten (PAH), flyktiga organiska ämnen (VOC) och partiklar. I Sverige har halten av föroreningsämnena i luften ständigt minskat, tack vare statens strängare utsläppsregler och nya miljövänligare bilar. Dock ökar samtidigt antalet bilar i landet och av denna anledning är det svårt att nå de uppsatta miljömålen.

Syftet med denna studie var att klargöra effekten av bilutsläpp på epifytiska lavar och att pröva hypotesen: det finns skillnad i mångfald av lavar på tallar mellan tre områden: (1) Väg 600 – en gammal väg som var huvudled mellan Uppsala och Gävle innan 2007, (2) E4:an – en ny motorväg mellan Uppsala och Gävle som invigdes under hösten 2007 och (3) ett referensområde som är opåverkat av luftföroreningar. Områdena är belägna i Tierps kommun.

Totalt hittades 24 lavarter och *Parmeliopsis ambigua* och *Hypogymnia physodes* var de vanligaste arterna i alla områdena. Medelartrikedomen var högst i referensområdet, men Väg 600 hade nästan lika hög medelartrikedom som referensområdet. Detta kan antagligen bero på den förbättrade luftkvaliteten. Däremot hade E4:an den lägsta artdiversiteten och detta kanske kan härledas ur den kraftiga ökningen av bilutsläpp. De resultaten antyder att vissa känsliga arter kan försvinna eller återkomma snabbt. Dock kunde detta påstående inte verifieras i denna studie på grund av inga tidigare inventeringsdata. Angående de tre luftkvalitetsindexmetoderna var de inte tillförlitliga för luftkvalitetsuppskattningen eftersom det fanns små skillnader i värden mellan de tre olika områdena. Kvävetal var inte heller den en relevant metod, för lavarna som hittades i alla områdena var inte kvävegynnande arter. Denna studie utfördes i skogen där även andra miljövariabler kan påverka mångfald av lavar. Dessutom hade Tierps kommun ett relativt låg luftföroreningsvärde. I en sådan miljö är det svårt att särskilja luftföroreningseffekten.

Abstract

Inventories of epiphytic lichens on Scots pine (*Pinus sylvestris*) were conducted at three sites in east central Sweden: along the newly build motorway (Road E4), along the old road (Road 600) and in a reference site, to compare the effects of traffic pollution on lichen diversities. At each site, species richness, lichen mean sensitivity value (MK), air quality index of the forest stand/sample plot (LKI) and nitrogen impact value (N) were calculated and it was tested if there was any difference between the sites. Twenty-four species were observed in total, and *Parmeliopsis ambigua* and *Hypogymnia physodes* were the most common species at all sites. At Road E4, fewer lichen species were observed than at the other sites and this might be due to the impact of air pollution. The species richness at Road 600 was similar to that of the reference site, which might be explained by the improved air quality. These results imply that some lichens may disappear or recover at fast pace with the change in pollution intensities. Yet, lack of previous lichen data prevented further analysis of the impact of air pollution on the lichen diversity. In terms of the air quality indices, they did not result in the expected pattern of better air quality at a lower-polluting site since the results were influenced by the only minor differences. Hence, the results of the air quality assessment based on these indices were not reliable in this study. Furthermore, the nitrogen impact value was not the relevant index since all the recorded species were acidophytes. The study area was a forest environment with high microenvironmental variables and a relatively low pollution level.

Keywords: bioindicator, air pollution, species richness, sensitivity, recolonisation, disappearance, forest ecosystem

1. Introduction

Lichens have a unique ecology in which a mycobiont and photobiont create a symbiotic relationship. The mycobiont is a fungus symbiont, mostly Ascomycota but sometimes Basidiomycota or Deuteromycota, while the photobiont is a green alga or cyanobacterium. Lichens can consist of one mycobiont and one or more photobiont, which means that this symbiotic entity may be composed of three kingdoms (Nash, 2008). Although lichens are often defined as symbiotic organisms, the relationships between mycobiont and photobiont are still incompletely known. For example, Ahmadjian (1993) claims that most of the benefits are gained by the mycobiont; the carbohydrates synthesised by the photobiont are mostly transported out of the cells instead of being used for its own growth. For this reason, the growth rate of the photobiont may be slower compared with that in the free-living condition. His study implies the requirement of further research on the relationships between mycobiont and photobiont.

Lichens are sensitive to their surrounding environment, not only due to the complex symbiotic relationships, but also due to their biological properties. Suitable habitat conditions and substrates are vital to the prosperity of lichens. Lichen species are often classified on the basis of the requirements of substrates, pH and ambient nutrient status (Seaward and Coppins, 2004). Therefore, studying lichen communities can illuminate the surrounding environmental change. Indeed epiphytic lichens have been recognised as indicators of air pollutions since the 1800s (Nash, 2008). Lichens are useful bioindicators, especially where technical instruments are not economically feasible (Seaward, 2008; Guidotti et al., 2009). Moreover, a correlation between air pollution and lung cancer in NE Italy by studying lichen biodiversity (Cislaghi and Nimis, 1997) suggests the potential use of lichens to monitor human health.

There is a tremendous amount of studies with respect to the effects of air pollution to epiphytic lichens and the use of lichens as bioindicators (Geebelen and Hoffman., 2001; Hultengren et al., 2003; Paoli et al., 2006; Davies et al., 2007; Giordani, 2007) as well as biomonitors (Nash, 1976; Herzig et al., 1989; Gombert et al., 2002; Frati et al., 2007; Tretiach et al., 2007; Riddell et al., 2008, Guidotti et al., 2009). Most of the studies have been carried out in urban areas where air pollution is caused by a number of factors. Though it is well-known that lichens are sensitive to air pollution, how fast lichens can be affected by air pollution and how fast they can recover from pollution has been poorly studied.

This study focuses on traffic air pollution and it aims at (1) clarifying the effects of air pollution caused by traffic emissions on corticolous lichen diversity and (2) investigating if there is any difference in the roadside lichen diversity on Scots pine *Pinus sylvestris* between a newly built motorway and an old road in east central Sweden. I test the hypothesis that fewer species occur at more polluted sites, resulting in a difference in lichen species diversity at the three sites. Based on the hypothesis, it will be discussed (3) how fast lichen communities can shift after a change in pollution intensity. Furthermore, (4) the validity of air quality indices will be evaluated. Scots pines have relatively acid bark and are favoured by certain epiphytic lichen species that prefer low pH. Pollutants emitted from vehicles such as nitrogen oxides can change the ambient nutrition status and may affect lichen community compositions. Hence, it might be possible to see the effects of air pollution by studying the species composition of lichens. An understanding of lichen recolonisation and disappearance can contribute to a more effective use of lichens as bioindicators of air pollution, but this was not in the scope of the current study.

1.1 Lichens as bioindicators to air pollution

Recognition of epiphytic lichens as bioindicators for air quality was addressed as early as in 1866 by the Scandinavian scientist William Nylander (Conti and Cecchetti, 2001). Since the 1970s, the use of lichens as biomonitors has been pronounced in a large number of studies.

There are several reasons why lichens are considered to be efficient and practical bioindicators. They are perennial and present all year round without changing morphology (Conti and Cecchetti, 2001; Guidotti et al., 2009). They are incredibly resilient and can be observed almost all over the terrestrial land (Seaward, 2008). Besides, they are slow growers and take their nutrients from the atmospheric deposition in the surrounding environment. Their direct absorption of water and nutrients from the air can simultaneously carry other substances including pollutants into the thallus (Guidotti et al., 2009). Furthermore, their sensitivity is enhanced by the lack of a protective cuticle and their exposed growth environment (Hultengren et al., 2004). However, the sensitivity can be influenced by different morphologies and biochemical properties. Nash (2008) points out that the sensitivity to different pollutants varies among different species. Moreover, Insarova et al. (1992) states that the response to different pollutants and substrates may vary even within a species.

Traffic emissions are one of the major environmental issues worldwide as a cocktail of pollutants is released into the air. For example, nitrogen oxides (NO_x), volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), particles and metals are some of the pollutants considered to be of ecological significance (Bignal et al., 2007; Bignal et al., 2008). In addition, effects of ammonia (NH_3) on vegetation at roadside verges (Truscott et al., 2005) and on bark pH that influences lichen vegetation around a pig stockfarm in Italy (Frati et al., 2006), and toxicity of nitric acid (HNO_3) to the lichen *Ramalina menziesii* (Riddell et al., 2007) have been recognised. Sulphur dioxide (SO_2) was once regarded as the most notorious pollutant affecting lichens, but the rapid reduction in SO_2 has been remarkable in the industrialised world today (Bates et al., 2001).

1.2 Sulphur dioxide

The relation of sulphur dioxide (SO_2) to lichens has been broadly studied throughout the world as this pollutant was once regarded as the most harmful compound to lichens. A wide range of methods is used to analyse the physical properties of lichens such as chlorophyll, sulphur isotope composition, sugar content, spectral reflectance, membrane proteins, moisture content and ethylene content (Conti and Cecchetti 2001). These studies have proved the deterioration of physical structures of lichens being exposed to sulphur compounds.

SO_2 is a pollutant produced by burning sulphur or any other compounds which contain sulphur such as coal and petroleum (Moor et al., 2008). SO_2 causes acid rain when it is oxidised with O_2 or ozone (Moor et al., 2008), which is one of the serious global issues in the world. In Europe, a high concentration of SO_2 in the air was prominent particularly in urban and industrial areas after World War II, and the maximum SO_2 emission was recorded in the 1960s and 70s (Mylona, 1996). Since the 1980s, the SO_2 concentration has decreased dramatically. In Sweden, the emission of SO_2 reached its peak in 1970s, with 962,000 tonnes per annum (Mylona, 1996). According to Statistics Sweden (SCB, 2010) the SO_2 emission was recorded 82,749 tonnes in 2008. The major emission sources are shipping companies, steel and metal works and pulp mills. The emission from motor vehicles accounts for 0.2% of the total amount of emitted SO_2 in Sweden (SCB, 2010). Svanberg and Lindskog (2000) point out that there are small differences in the SO_2 load between urban and rural areas today.

According to Swedish Environmental Protection Agency (EPA, 2011), the environmental quality standards for ambient air quality were introduced by the Swedish Environmental Code in 1999. The standard for sulphur dioxide is described below (Table 1). The concentration has been under the threshold of the standard in Sweden since 1990 (Stockholm – Uppsala County Air Quality Management Association, 2010). Moreover, the interim target in which an annual mean level of SO_2 should not exceed 5 µg/m^3 has been fulfilled by 2005 in all municipalities (EPA, 2011).

Table 1. Environmental quality standard for concentrations of sulphur dioxide established by the Swedish Environmental Code for air quality (2010: 477). The limit values are set for prevention of harmful effects on public health and vegetation.

a. Public health

Averaging time	Mean value (µg/m^3)
1-hour	200
24-hour	100

b. Vegetation

Averaging time	Mean value (µg/m^3)
Winter mean value (1 Oct - 31 Mar)	20
Annual	20

Source: "Luftkvalitetsförordning" (2010 : 477) (the Swedish Environmental Code for air quality) by Miljödepartementet (Ministry of the Environment)

1.3 Nitrogen oxides

Nitrogen oxides (NO_x, NO and NO_2) and ammonia comprise the major sources of atmospheric nitrogen deposition (Truscott et al., 2005). Nitric oxide (NO) is an unstable chemical compound generated e.g. in combusting fossil fuels in motor vehicles (European Bioinformatic Institute, 2011). The emitted NO is converted rapidly to nitrogen dioxide (NO_2) in the air by the oxidisation process (Moor et al., 2008). In contrast to SO_2, NO_2 concentration in the air has increased or been steady over time in most of the industrialised nations due to an increased volume of traffic. NO_2 is a toxic air pollutant that causes a number of health problems in humans and can be harmful to the surrounding vegetation. For example, inhibition of plant growth and defoliation of plants in NO_2 concentration of 25-250 ppm are proved in laboratory tests (Moor et al., 2008). Bignal et al. (2007) point out that the effect of NO_2 emitted from road transport on local vegetation can be observed up to a distance of 100 m from the road.

Many recent studies relating to the effects of air pollution on lichens focus on NO_x and there are numerous results showing the significant correlation between lichens and the pollutant.

Nitrogen is an essential element for life, being involved in the synthesis of protein and nucleic acids (Nash, 2008). However an excess amount of nitrate deposition can deteriorate the symbiotic relationship. For instance, NO_x has a strong effect on lichen diversity (Davies et al., 2007), its community composition, frequency and dispersal (Larsen et al., 2007). In addition, lichen population declines in high NO_x content (van Dobben et al., 2001; Giordani, 2007). At the molecular level, Tretiach et al. (2007) showed that a large amount of NO_x can damage the photobionts of transplanted *Flavoparmelia caperata*, hindering photosynthesis. This is probably due to the increased reactive oxygen species (ROS). They reported that a high concentration of NO_2 in the cells forms nitrous and nitric acid, which acidifies cytoplasm and results in protein denaturation, deamination of amino acids and nucleic acid.

Despite those negative effects, some lichen species such as *Lecanora dispersa* and *Phaeophyscia orbicularis* are NO_x tolerant (Davies et al., 2007). Even though Nash (1976) confirmed in a laboratory experiment the phytotoxic effect of NO_2 on lichens that were fumigated with 4 ppm (7520 $\mu g/m^3$) for six hours, he suggested that the pollutant would probably not be harmful to lichens since the NO_2 concentration detected in natural environment was usually less than 1 ppm. Thus, the effect of NO_x on lichens seems controversial and unclear.

In Sweden, total NO_2 emissions have decreased since the 1980s owing to a reinforced regulation on exhaust gas (EPA, 2011). Simultaneously, increase in traffic keeps the NO_2 emission stable. The total emission of NO_2 was 310,961 tonnes in 2008 (SCB, 2011). The environmental quality standard for nitrogen oxides in Sweden is shown in Table 2. The EPA's goal that the average hourly and yearly concentrations should be less than 60 and 20 $\mu g/m^3$, respectively, in 2010 was not achievable mainly due to the high NO_2 concentration in the areas with high traffic density.

Table 2. Environmental quality standard (a) for mean hourly, daily and yearly nitrogen dioxide (NO_2) values and (b) for average yearly nitric oxide (NO) value given by the Swedish Environmental Code for air quality (2010: 477). The limit values for prevention of harmful effects on (a) public health and (b) vegetation.

a. Public health

Averaging time	NO_2 mean value ($\mu g/m^3$)
1-hour	90
24-hour	60
Annual	40

b. Vegetation

Averaging time	NO mean value (µg/m^3)
Annual	30

Source: "Luftkvalitetsförordning" (2010 : 477) (the Swedish Environmental Code for air quality) by Miljödepartementet (Ministry of the Environment)

1.4 Ozone

Ozone (O_3) is a secondary pollutant as a result of the chemical reaction of O_2, NO_2 and oxygen-containing organic compounds (Moor et al., 2008). The content of O_3 in the air is variable since it is influenced by weather conditions, seasonality, times of day and changes in the amount of long transported O_3 (EPA, 2011). In the arid zones of the USA where petrol is heavily combusted, O_3 can be a significant pollutant (Riddell et al. 2008). In Sweden, the interim target for the ground-level ozone (Table 3) by 2010 was not achieved and furthermore, it can be difficult to reach the standard in 2011.

Table 3. Environmental quality standard for concentrations of ground-level ozone established by the Swedish Environmental Code for air quality (2010: 477). The limit values are set for prevention of harmful effects on public health and vegetation.

a. Public health

Averaging time	Mean value (µg/m^3)*h
8-hour	120

b. Vegetation

Time	Mean value (µg/m^3)*h
May-July	18000^a
May-July	6000^b

^{a} mean value in 5 years, until 31/12/2019

^{b} mean value in 5 years, from 01/01/ 2020

*Source: "Luftkvalitetsförordning" (2010 : 477) (*the Swedish Environmental Code for air quality*) by Miljödepartementet (Ministry of the Environment)*

The effects of O_3 on lichens are poorly studied, compared with SO_2 and NO_2. The studies of toxicity of O_3 to lichens seem controversial. O_3 causes oxidative damage of cell membranes in lichens as a result of peroxidation of lipid membrane (Conti and Cecchetti, 2001). Moreover, a field study with transplanted *Hypogymnia physodes* by Egger et al. (1994) showed a high amount of end-products of peroxidation in lichens exposed to a high concentration of O_3. However, Riddell et al. (2010) found no negative response of *Ramalina menziesii* to O_3 fumigations.

1.5 Other air pollutants

In addition to the pollutant compounds mentioned above, the interim targets for volatile organic compounds (VOCs), benzo(a)pyrene and particulate matter (PM) are also included in the air quality assessment in Sweden (EPA, 2011). Emissions of VOCs in Sweden have declined largely and the targeted goal was accomplished by 2010 (EPA, 2011). Benzo(a)pyrene is a representative of Polycyclic aromatic hydrocarbons (PAHs). The goal that an annual mean level of benzo(a)pyrene should not exceed 0.3 µg/m^3 would be reachable by 2015 if further action is taken (EPA, 2011). As to particles, the targeted levels of particles (PM10 and PM2.5) are less likely to be reached. An exceeded concentration of particles is recorded in many large cities; the major sources are the use of studded tires, traffic emissions, combustion of wood for heating and long-distance transport to Sweden from other European countries.

Lichens as biomonitoring or bioaccumulating organisms for those pollutants are very poorly studied. Yet the possible use of some lichen species as biomonitors for the concentration of PAHs have been recognised (Guidotti et al., 2009;Shukla and Upreti, 2009).

2. Material and Methods

2.1 Study location

The field work was carried out in May 2011. There were three study localities: (1) along the newly opened motorway (Road E4), (2) along the old road (Road 600) and (3) at a reference site which is in a forest area and totally unaffected by air pollution (Fig. 1). All three study sites were located within Tierp municipality, approximately 130 km north of Stockholm. The mean annual precipitation and temperature in the area is 600–700 mm and 5–6°C, respectively (Swedish Meteorological and Hydrological Institute, 2011).

The transect along the motorway E4 is located approximately 4 km south of central Tierp (Fig. 1). It is part of the new motorway from Uppsala to Gävle opened in October 2007. The transect along the road 600 is located between Tierp and the small village Mehedeby, about 6 km north of central Tierp (Fig. 1). This road was presumably built in the 17th century when the road between Uppsala and Björklinge was constructed for the queen Kristina (Samuelsson, 2011). Road 600 was the main road between Uppsala and Gävle before the motorway was built. Table 3 shows the average number of vehicles passing Road E4 and Road 600 in the surveyed area each day (Samuelsson, 2011). Of the total number of vehicles, the heavy vehicles account for 10% on both roads. Six times more vehicles are recorded on the motorway E4 than on the road 600. The road 600 probably had as heavy traffic as the motorway E4 before the motorway was opened (Samuelsson, 2011) but unfortunately there are no detailed records available.

The reference site is located in a community Djupa, approximately 10 km southwest of Tierp (Fig. 1). The first plot is placed at 50 m south from the gravel road and the transect continued southwards.

Table 3. Average number of motor vehicles that pass E4 and road 600 per day (Samuelsson, 2011).

	E4	600
Light vehicles	11700	1980
Heavy vehicles	1300	220
Total	13000	2200

Source: Trafikverket (the Swedish Transport Administration)

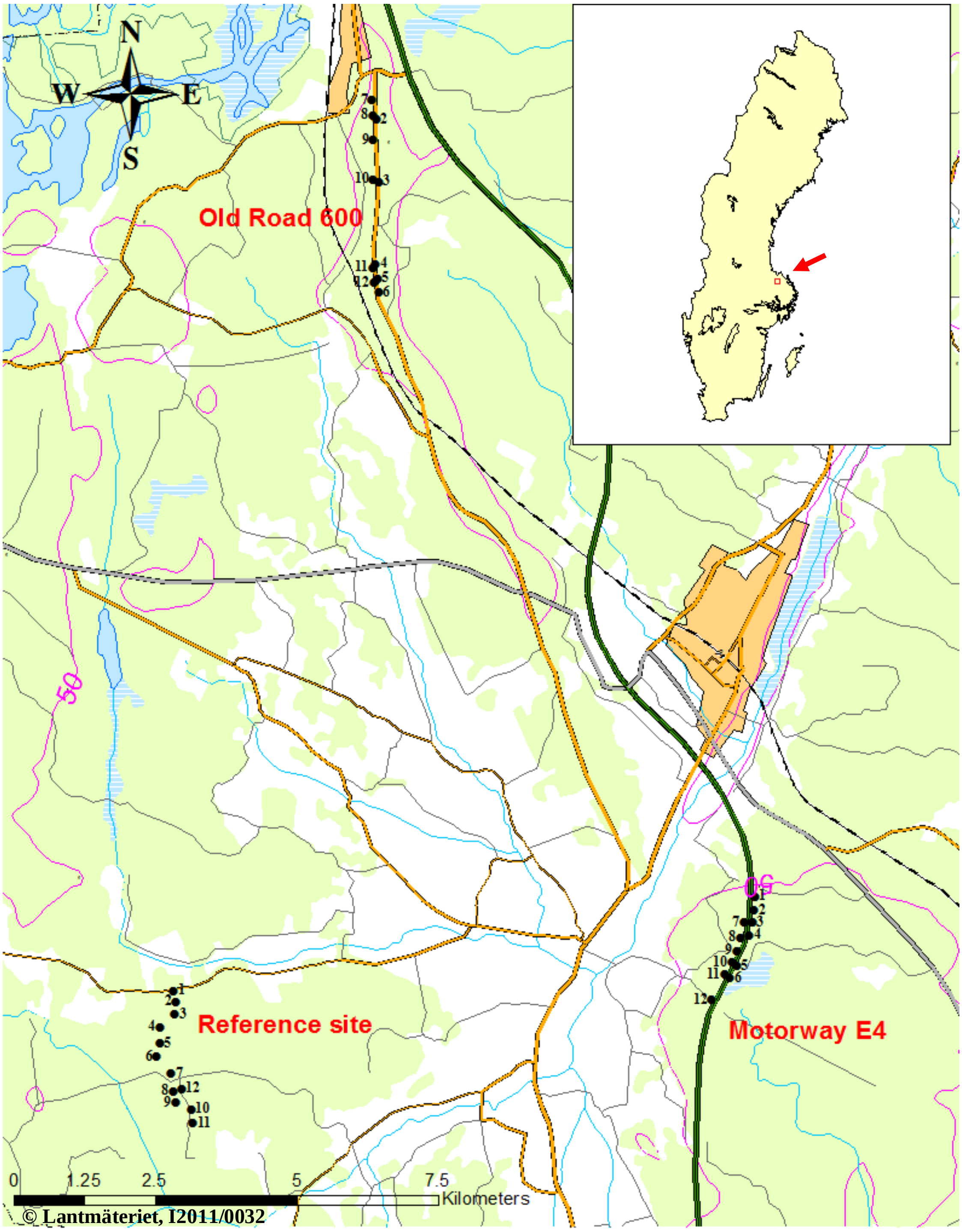

Fig. 1. A georeferenced map showing three inventory sites and the twelve sampling plots* at each site, and a background map of Sweden showing the study location. The three study sites are located in Tierp municipality in Uppsala County, east central Sweden.

*GPS coordinates for Plot1 at Road 600 is missing.

Source: Digitala kartbiblioteket, Lantmäteriet.

Software: ArcGIS

Projection: SWEREF99

Air quality around the survey area

The data on nitrogen dioxide concentration at the study area was obtained from Stockholm – Uppsala County Air Quality Management Association (LVF, 2011). Fig. 2 shows 98-percentile mean annual values of NO_2 background concentration in 2009. The mean NO_2 level at Road E4 was 7–11 µg/m^3 whereas Road 600 had 3–5 µg/m^3. The reference site had 3–4 µg/m^3 and was unaffected from the traffic pollution.

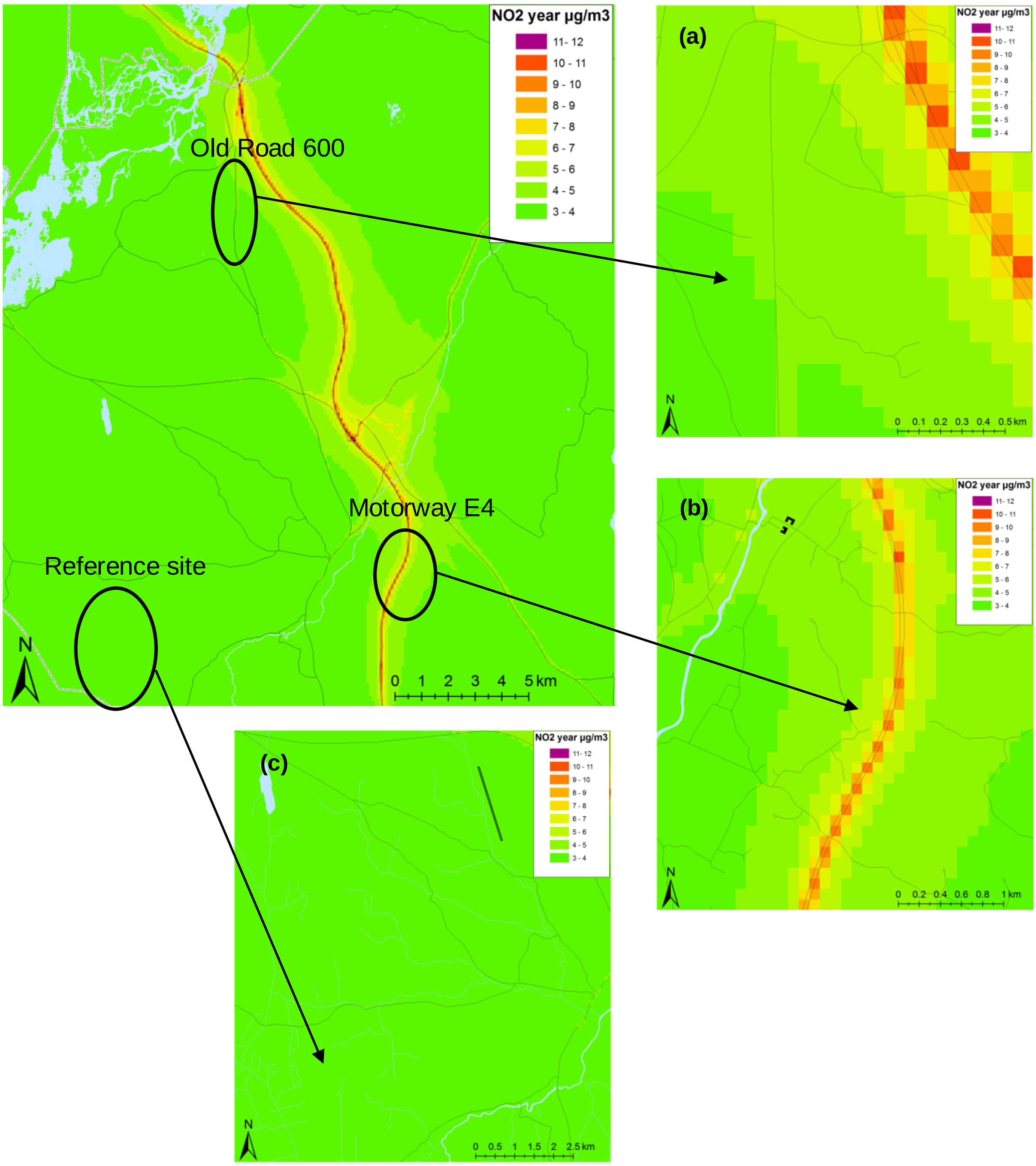

Fig. 2. A raster map over the survey area and three enlarged maps for (a) Road 600, (b) Road E4 and (c) reference site illustrating the mean daily values (24 hours) of nitrogen dioxide concentration. Each pixel represents the actual area of 100 x 100 m. *Source: Stockholms – Uppsala County Air Quality Management Association*

2.2 Inventory

The lichen inventory was conducted in semi-open conifer forests dominated by Scots pine *Pinus sylvestris* L. Barkman (1958) claims that bark chemistry is one of the factors that affect lichen communities and it varies in different tree species. Furthermore, the study by Van Dobben et al. (2001) points out that lichen species composition of the vegetation can be determined by a natural variation like bark pH, tree species and tree DBH. These criteria are therefore important to make observation as homogenous as possible. In this study only the lichen flora on a single tree species, Scots pine, is studied to minimize the variables caused by bark physiology. Based on this concept, Hultengren et al. (1992) divided common tree species occurring in Sweden into five groups depending on the nutritional conditions and pH of the bark. According to this classification, Scots pine belongs to “Group 2”, characterised by poor nutritional conditions and low bark pH.

To examine the effect of air pollution only from road traffic, it was crucial to avoid the area that could potentially be exposed to the effects from other pollutants, e.g. from industries, residential areas and arable fields. The survey area is located in a rural environment, since urban environments are affected by a complexity of various pollutants emitted from different sources (Frati et al., 2006). The transect along Road E4 and along Road 600 consisted of twelve plots, each 20 m × 20 m with six on the east and six on the west side of the road. The transects were placed adjacent to the road, where a tree line started. The transect along Road E4 is 2.1 km in length and Road 600 stretches approximately 3.5 km. For the reference site, twelve plots were placed from north to south along an approximately 2.4 km long transect. The centre of each plot was marked with GPS (Garmin 60CSx) with a maximum accuracy level of ±5m. In order to obtain the knowledge on the diversity of the lichen flora along the entire transect, the plots were laid at 250 m intervals. The intervals were extended when the suitable Scots pine forest for the inventory was not found. At each plot, the three closest pine trees from the centre of the quadrat were chosen. These trees should have a diameter of 25–35 cm (circumference 78–110 cm) at 130 cm above the ground and grew in semi-open forest with no deciduous trees growing close by.

The presence of all foliose and fruticose lichen species on each sampled tree was recorded between 50 and 200 cm above ground. For crustose lichens, only those common species that were included in the report by Hultengren et al. (1992) and that were easy to be indentified were inventoried. All the samples that were unidentifiable in the field were collected and brought to the laboratory for species identification.

2.3 Indices and data analysis

The obtained field records were analysed using sensitivity classification and lichen bioindicator indices by Hultengren et al. (1992), which are based on their own experiences and the relevant literature. Since classification was developed from observations in Sweden, they are particularly suited for the Swedish environment. Hultengren et al. (1992) describes the sensitivity values of 52 selected species. These species are easily identified and commonly observed, and therefore can be used as indicator species to assess air quality. Several species that were found in this study were not among the 52 species and thus they were excluded in some index calculation described below. However all the recorded species were important in assessing the species richness.

Number of species/tree (M)

The total number of lichen species found on each tree was summed and the mean number of species per plot was calculated. All the recorded species were included in this model.

$$\mathrm{M} = 1/t \sum_{i=1}^{t} m_i$$

m_i = total number of species per tree
t = number of trees

According to Hultengren et al. (1992), this calculation is simple and practical on the field as the information is easily collected without any special equipment and deep knowledge of lichens. Furthermore, the obtained values are objective. On the other hand, the model gives equal value to all species, no matter how sensitive or tolerant they are.

Lichen frequency

The frequency of each species (occurrence) was calculated for the two road sites and the control site.

$$\text{Species frequency (\%)} = \frac{Number\ of\ trees\ on\ which\ respective\ species\ is\ present}{Total\ number\ of\ observed\ trees} A?\ 100$$

Lichen sensitivity (K)

Sensitivity values of the selected lichen species ranged from 0 to 9 and were according to Hultengren et al. (1992) (Table 8). The more sensitive to air pollutions a species is, the higher the value is. For instance, an area in which species with high sensitivity values are found has high air quality. The definition of each value is shown in Table 4.

Table 4. Sensitivity values and the description for each value according to Hultengren et al. (1992).

Value	Sensitivity to air pollution
9	Very sensitive
8	Very sensitive
7	Very sensitive
6	Very sensitive
5	Sensitive
4	Sensitive
3	Tolerant
2	Tolerant
1	Very tolerant or pollution-favoured
0	Very tolerant or pollution-favoured

Mean sensitivity value (MK)

On the basis of the lichen sensitivity described above, mean sensitivity value for each plot was calculated with the following formula:

$$\mathrm{MK} = 1/n\sum_{i=1}^{n}\left(K_i\right)$$

K_i = respective species sensitivity
n = number of species

This model is a compromise between different index calculations (Hultengren et al., 1992). It is easy to calculate and has little consideration to tree types and geographic region. It is important to ensure that all or almost all the species present in an area are recorded in order to obtain the most accurate result.

Air quality index of the forest stand/sample plot (LKI)

This formula uses the sensitivity value and species frequency as observation per tree stand. Tree species should be taken into consideration when comparing the calculated values: the values from different tree species should not be compared. Hultengren et al. (1992) states that this index is also a compromise between various index calculations. It calculates a relative frequency that takes the number of observed species into account and less consideration to the shapes of thalli. Thus, the model can avoid mistakes associated with the coverage calculation that is commonly used worldwide.

$$\mathrm{LKI} = 1/t \sum_{i=1}^{n} \left(K_i \times f_i\right)$$

K_i = respective species sensitivity
f_i = number of trees where respective species is observed
t = number of inventoried trees
n = number of species

Nitrogen impact value

This classification is also derived from Hultengren et al. (2010) and is based on the study by Wirth (1980). Each of the selected species is rated in accordance with its preference of nitrogen (Table 5). Then the mean nitrogen impact value on each tree is averaged from the recorded species. The proportion of nitrogen-preferring lichens on a tree depends on the nutrient status of the bark and the effect of nitrogen-related air pollution. In general, trees with poor nutrients such as birch and pines have low nitrogen impact values in an unpolluted environment and high in an environment with high nitrogen pollution. In other words, a high value on such a bark indicates that the area is highly affected by traffic air pollution or fertilizers or eutrophicated dust from agricultural practices.

Table 5. Nitrogen impact values (N) and the description of each of the seven categories according to Hultengren et al. (2010)

N value	Category
3	Highly nitrogen-preferred
2.5	Relatively to highly nitrogen-preferred
2	Relatively nitrogen-preferred
1.5	Slightly to relatively nitrogen-preferred
1	Slightly nitrogen-preferred
0.5	Not nitrogen-preferred to slightly nitrogen-preferred
0	Not nitrogen-preferred

Statistical analysis

A parametric test General Linear Model (GLM) in ANOVA was used to evaluate if there was any significant difference in the total number of species and MK between three untransformed sample data: Road E4, 600 and Reference sites. As an ANOVA post hoc test, Tukey’s test was selected to determine whether any two pairs of means are significantly different from each other. For the comparison of LKI, a non-parametric Kruskal-Wallis test was applied since the observations were not normally distributed. As a post hoc test, non-parametric Tukey-type multiple comparison (Zar, 1984) was used and calculated manually with Excel.

ANOVA, Kruskal-Wallis test and Tukey’s test were performed using the statistical programme package Minitab 16. Species accumulation curves are produced by using EstimateS Version 8.2 (Colwell, 2009) in order to estimate the species richness at each site and to answer the question ‘does this survey reflect the sufficient coverage of species?’.

3. Results

3.1 Species richness (total number of taxa)

A total of 24 lichen species were recorded (Table 8) and the total species number for Road E4, Road 600 and the reference site were 18, 23 and 21 respectively. The mean number of lichen species was highest at the reference site and lowest at Road E4 (Table 6). The largest species number in single plots was 17, recorded at one plot at Road 600. The lowest species number noted was six, observed in plots in all the transects. The GLM analysis showed that the mean number of taxa was significantly different (F = 4.07, P = 0.026) between three sites (Table 7 & Fig. 3).

The most common species at all sites was *Parmeliopsis ambigua* with 100% of occurrence followed by *Hypogymnia physodes*. Except these species, the occurrence of the other species was different at each site. The most commonly found species along Road E4 were *Lecidea pullata* (83% of all plots) and *Vulpicida pinastri* (78%), whereas *Vulpicida pinastri* (58%), *Parmeliopsis hyperopta* (56%) and *Hypocenomyce scalaris* (56%) were the most common noted species along Road 600. At the reference site, *Lecidea pullata* (89%), *Parmeliopsis hyperopta* (69%) and *Vulpicida pinastri* (58%) were frequently observed. The highly pollution-sensitive fruticose lichens, *Usnea filipendula* and *Bryoria* spp, were absent at Road E4.

Table 6. Total number of species recorded in each plot (1-12) and the mean number in the three transects.

Site	1	2	3	4	5	6	7	8	9	10	11	12	Mean
E4	7	9	7	7	10	9	4	6	9	9	8	7	7.7
600	9	11	7	11	8	11	8	6	9	9	8	17	9.5
Reference	10	10	11	8	12	12	11	6	9	12	10	10	10.1

Table 7. The results of the General Linear Model analysis for the total number of species at Road E4, Road 600 and the reference site. DF (Degree of freedom), Seq SS (Sequential sums of squares) Adj SS (Adjusted sums of squares), Adj MS (Adjusted mean squares)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	38.167	38.167	19.083	4.07	0.026
Error	33	154.583	154.583	4.684		
Total	35	192.75				

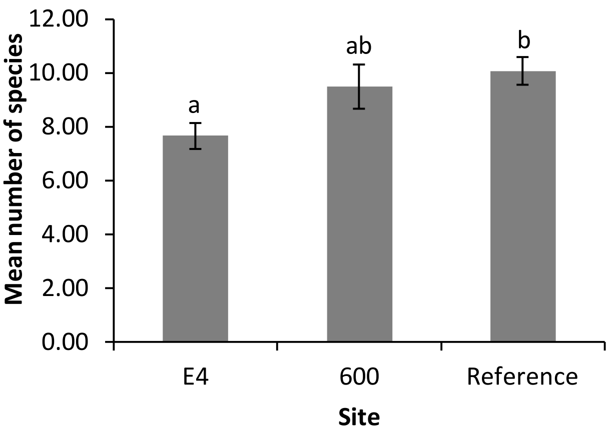

Fig. 3. Mean number of lichen species at Road E4 (motor way), Road 600 (old road) and the reference site. Error bars represent standard errors (±). The same letters above means of each sample indicate that there is no significant difference between the samples (Tukey's test).

Table 8. Total number of observed species and their sensitivity value and nitrogen value according to Hultengren et al. (1992) with the occurrence of each species per site. Hyphen mean no data available.

			Occurrence %		
Species	Sensitivity	Nitrogen	E4	600	Reference
Usnea filipendula	7	0	0	11	11
Imshaugia aleurites	7	-*	28	50	19
Mycoblastus sanguinarius	7	-	6	8	0
Bryoria fuscescens	6	0	0	3	3
Bryoria capillaris	6	0	0	0	14
Hypogymnia tubulosa	5	0	3	17	0
Hypogymnia farinacea	5	0	8	19	3
Platismatia glauca	4	0	6	31	6
Pseudevernia furfuracea	4	0	3	44	0
Vulpicida pinastri	4	0	78	58	58
Parmeliopsis hyperopta	3	-	42	56	69
Cladonia coniocraea	2	0	0	22	31
Hypogymnia physodes	2	0.5	100	100	92
Parmeliopsis ambigua	2	0	100	100	100
Hypocenomyce scalaris	2	0	6	56	31
Lepraria incana	1	0	31	6	36
Cladonia digitata	-	-	3	11	19
Lecidea pullata	-	-	83	50	89
Cladonia chlorophaea	-	-	17	8	19
Cladonia cenotea	-	-	11	6	42
Cladonia pleurota	-	-	6	6	14
Cladonia ochrochlora	-	-	0	6	11
Cladonia deformis	-	-	0	3	8
Ochrolechia microstictoides	-	-	8	3	3

The species accumulation curves are shown in Fig.4. Road 600 and the reference site had the similar pattern at the initial slopes, whereas the slope for the Road E4 was more gradual than the other two sites. The accumulation curve for the reference site levelled off earlier than that of Road 600.

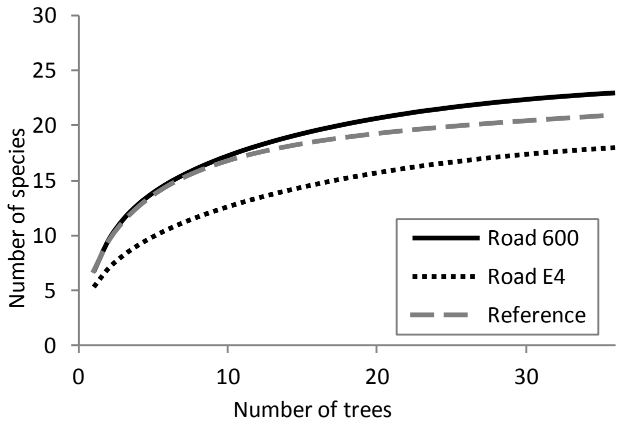

Fig. 4. Species accumulation curves for three inventoried sites generated by using the analytically calculated Sobs (Mao Tao). Each site is comprised of 12 plots and three trees in each plot: 36 trees per site.

3.2 Mean sensitivity value

Of the 24 observed species, the number of the species which were given the sensitivity values by Hultengren et al. (1992) were 16. Hence the mean sensitivity value (MK) for each plot was calculated with these selected species (Table 8). The average MK was higher at Road 600 than the other sites (Table 9). Both the highest and lowest MKs were found at the reference site, 3.72 and 1.83 respectively. The average MK for Road E4 and the reference site indicates that both sites are moderately affected by air pollution (Table 9 & 10). Road 600 was within the category 'weakly affected'.

Table 9. Mean sensitivity value (MK) per plot (1-12) and per site. Each value is the averaged value from three observed trees in each plot. The mean MK is the average value from the twelve plots per site.

Site	1	2	3	4	5	6	7	8	9	10	11	12	Mean
E4	3.65	3.14	3.09	3.08	2.86	3.08	2.67	2.69	2.56	2.36	2.04	2.78	2.83
600	3.61	3.56	3.24	3.55	3.28	3.04	3.06	2.67	3.60	2.25	3.39	3.37	3.22
Reference	3.45	3.18	3.20	3.72	2.92	2.55	2.59	1.83	3.06	2.16	2.58	2.53	2.81

Table 10. Interpreted effects of air pollution on lichen flora according to the mean sensitivity values by Hultengren et al. (2010). The higher the MK is, the less lichen flora is affected by air pollution.

MK	Status of lichen flora
>4	completely unaffected
>3-3.9	weakly affected
>2-3	moderately affected
1-2	strongly affected
>1,0	very strongly affected and depleted

The GLM analysis indicated that there was a significant difference between the means of MK of three sites (F = 3.84, P = 0.032) (Table 11 & Fig.5).

Table 11. The results of the General linear model (GLM) for mean sensitivity values at Road E4, Road 600 and the reference site. Each site consists of data from 12 plots. DF (Degree of freedom), Seq SS (Sequential sums of squares) Adj SS (Adjusted sums of squares), Adj MS (Adjusted mean squares)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	1.4647	1.4647	0.7324	3.84	0.032
Error	33	6.2987	6.2987	0.1909		
Total	35	7.7634				

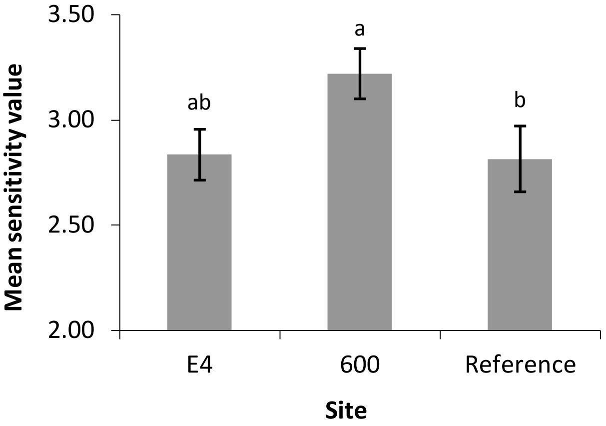

Fig. 5. Average MK at Road E4, Road 600 and the reference site. Error bars represent standard errors from the mean values (±). Samples which shares the same letter above means indicate that the means are not significantly different from each other (Tukey's test).

3.3 Air quality index of the forest stand/sample plot (LKI)

Same as the MK described earlier, the calculation of LKI was performed with the 16 species (Table 8). The highest LKI was 26.7 at Road 600 and the lowest 8.0, found at both Road E4 and the control site (Table 12). The results of Kruskal-Wallis test are shown in Table 13 and Fig. 6. The P-value of 0.003 indicates that there was a significant difference between three transects. Based on the multiple-comparison test, Road E4 was significantly different from Road 600.

Table 12**.** Lichen air quality index at each plot (1-12) of Road E4, Road 600 and the reference site. Each value was the average of LKI for three observed trees.

Site	1	2	3	4	5	6	7	8	9	10	11	12	Mean
E4	17.0	15.0	11.0	12.3	15.3	16.0	8.0	9.0	9.3	8.7	9.7	11.3	11.9
600	19.3	25.7	15.3	26.7	15.3	23.7	19.3	11.7	25.3	10.7	16.0	24.3	19.4
Reference	15.0	14.3	16.0	16.3	17.0	14.0	14.7	8.0	14.0	10.3	12.0	8.3	15.4

Table 13. The results of the Kruskal-Wallis test for lichen air quality index at Road E4, Road 600 and the reference site. N = number of observations, Z-value = to determine how the mean for each group differs from the mean of all observations.

Site	N	Median	Ave Rank	Z	P
E4	12	11.17	12.8	-2.3	0.003
600	12	19.33	26.8	3.32	
Reference	12	14.17	16	-1.02	
Overall	36		18.5		

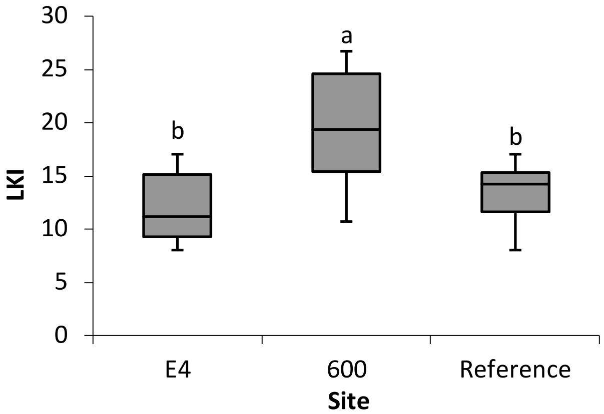

Fig. 6. Box Plot displays the statistical summary of the lichen air quality index (LKI) at three study areas. Samples that have the same letters above upper whiskers indicate that the medians are not significantly different from each other.

Regarding the nitrogen impact value, all the species had the value 0, except *H. physodes* (0.5) (Table 8). This indicates that all the species were categorised into the same group that did not favour nitrogen (Table 5). Therefore, this index was not a relevant measurement for the comparison between the three sites, and further statistical analysis was not performed with the result of the nitrogen impact value.

4. Discussion

Air pollution from traffic emissions is an ongoing global issue, since the emitted pollutants can deteriorate the surrounding environment and cause human health problems. Despite the improved air quality in Sweden over the past few decades, the continuously increasing traffic density hampers further reduction of environmental impacts. The impact of traffic pollution on the vegetation of the adjacent roads is still significant in heavily travelled areas (Truscott et al., 2005; Bignal et al., 2007, Bignal et al., 2008) and thus it needs to be carefully investigated.

4.1 Species richness and community composition

A remarkable difference was found in lichen species richness between Road E4 and the reference site, and it is possibly associated with traffic pollution. Interestingly, the mean number of species at Road 600 was almost as large as at the reference site. Moreover the highest total number of species per site was recorded at Road 600 with 23 species. This implies that its habitat quality is similar to the reference site, or in some cases the site may be more favourable to some lichens. Yet, the result is based only on the number of recorded species and it might not be feasible to draw a solid conclusion. Concerning the species composition, foliose lichen *Imshaugia aleurites* which is classified as highly sensitive lichen was present at Road E4 at the frequency of almost 30%. This result suggests that higher number of species may not reflect better air quality, but the sensitivity of *I. aleurites* may be overestimated. In general, fruticose lichens are most susceptible to pollutions, whereas crustose lichens are most tolerant and foliose lichens are intermediate. Therefore fruticose lichens should have higher sensitivities than foliose lichens. The absence of fruticose lichens *Usnea filipendula* and *Bryoria* spp. at Road E4 suggests that the area was more polluted than the reference site.

Ellis and Coppins (2009) found that air pollution gradients were highly correlated to the patterns of epiphytic lichen composition, but scarcely explained the variation in species richness in the area with low-to-moderate pollution loads. Furthermore, it has been revealed from a study in severely polluted regions in Netherland that the species composition has changed from acidophytic to nitrophytic communities due to decrease in SO_2 pollution and increase in NO_2 pollution (van Herk, 1999). His study points out that species composition can change completely, though the number of species may remain the same. Therefore studying only species richness can be misleading and it is of importance to study which species comprise the community in the study area. According to the nitrogen impact value assessment, the examined species (13 species) were all acidophytes and any compositional difference was not found in terms of the nitrogen value. Yet, the result of lichen diversity can still be worth considering, since the sensitivity to air pollution varies among these acidophytes. The lowest species number and absence of fruticose lichens at Road E4 can be a reflection of the negative environmental effects.

Many species were observed on both living and dead tree twigs that were not included in this inventory. Particularly at the reference site, some fruticose species with high sensitivities were present on branches. It is difficult to assess the impact of microclimatic variations on the results in this study. Although the study sites were all located in the same climate zone, the complex microclimatic variables could have affected the dynamics of lichen flora. The exclusion of several fruticose and foliose lichens observed on twigs was due to the different biotope from the trunks. Both should be included in the future study to obtain more accurate data of epiphytic lichen diversity.

The result of species accumulation curves suggests that the species richness at Road 600 could be higher than the reference site. Although the shapes of the curves for the two sites are similar, the curve for the reference site is predicted to reach the asymptote earlier than Road 600. This indicates that the diversity of unique species can be higher at Road 600. Light is one of the significant factors for lichen colonisation. Perlmutter (2010) states that the trunk lichen flora of forest trees generally host a reduced lichen flora because of its shady environment. Road 600 is adjacent to a glade (road) may allow more light to enter the forest. Since there is little impact of air pollution at Road 600 today, the availability of light may allow many lichen species to colonise. Yet, it is difficult to assess this factor. If the light was the major factor, the similar lichen flora should have been observed at Road E4. Comparing the lichen flora close to the clear cut with that surrounded by forest may be one option to assess the effect of light. In summary, the difference in the slopes of two curves is very small. Together with the GLM analysis, it is reasonable to conclude that Road 600 and the reference site had a similar environmental quality.

4.2 Air quality assessment

Surprisingly, the index analyses indicated that the mean sensitivity value was higher at Road 600 than at the reference site, suggesting that Road 600 had the best air quality. There are numbers of calculation models in respect to air quality index using observation of lichens and this study used the mean sensitivity value and air quality index of the forest stand/sample plot. Both indices are practical and straightforward with basis of the recorded number of species. However, as mentioned earlier, Hultengren et al. (1992) suggests that all species should be recorded to obtain the reliable mean sensitivity value in a region. The inventory was conducted on three trees per plot along the three transects. This means that the recorded lichens probably did not represent all species which could be present at each site. Thus the resulted mean sensitivity value may bias the actual air quality to some extent. This can be related to the result of the reference site that is located in a remote area and should have no impact of traffic pollution. Nevertheless, the average mean sensitivity value indicates that the area was moderately affected by air pollution. A similar result was obtained in a study by Segerlind (2009). This is probably due to the little difference in the values at the three sites. On the other hand, Segerlind (2009) suggests that the historical aspect should be considered. He states that the low mean sensitivity value at the reference site was associated with the altered environmental condition due to the road construction more than 20 years ago. The reference site in this study was the forestry area. The construction of forestry path for machineries as well as forest management can largely influence the forest environment. Therefore the potential impact of forestry practice may have led to the low mean sensitivity value at the reference site. In this case it is extremely difficult to judge the air quality with the mean sensitivity value. Road E4 and Road 600 are subjected to the synergetic effects of traffic emissions and forestry. Air quality index of the forest stand/sample plot, on the other hand, can be a more precise method. It assesses the air quality by taking the sensitivity of each recorded tree stand into account. However, even the lichen flora of the three selected trees in the same plot were heterogeneous in many plots, which means that the result could largely be biased by the selection of trees and it can be difficult to standardise the result. Nevertheless, better air quality at Road 600 than Road E4 by comparing the two sites (Fig. 5 & 6) is well correlated to the pollution loads for each site (Fig. 2).

4.3 Rapid recolonisation/ disappearance

Lichens are able to recolonise quickly when the air quality improves (Ahmadjian, 1993), but it is rarely known how fast they can return to the environment and if there is any species difference in terms of recolonisation rate. Many studies show the recolonisation of lichens after a long time period, often longer than 10 years (e.g. Hultengren et al., 2003; Davies et al., 2007; Lisowska, 2011). Besides, there are few studies of their disappearance rates associated with air pollution.

Lichens are generally considered to be slow-growing and slow-colonising (Hultengren et al., 2003). On the other hand, annual change of lichen communities as a result of declined concentration of air pollutants is reported (Loppi et al., 2004). If the lichen flora at Road 600 before autumn in 2007 was similar to that of Road E4 today, a rapid comeback of some lichens due to the considerable decrease in traffic flow after the motorway opening have taken place. Yet, a lack of previous inventory data and other data such as the on-site air quality data impedes further analyses and judgements. It is interesting to investigate at what pace lichens can recolonise and if there is any interspecific difference in the recovery rate.

According to the air quality assessment by the Stockholm – Uppsala County Air Quality Management Association (2011), there was little difference in NO_2 concentration between Road E4 and the reference site before the motorway was opened. This indicates that the area was unpolluted. Presuming that lichen diversity in the area was similar to the reference site, some species may have disappeared at a rapid rate within a few years. Today, the concentration of the pollutant is approximately twice as high along E4 as the reference site (Fig. 2). This rapid change in air quality and other disturbance factors related to the road construction and the forestry practice can be linked to the presumed rapid disappearance of lichens at Road E4. Particularly the impact of road construction including forest removal on both abiotic (e.g. light and water sources) and biotic changes in the surrounding environment should be considered.

4.4 Low pollution load and forest ecosystem

Despite the distinctive difference in the results between Road E4 and Road 600, it may be difficult to consider the traffic pollution as the most significant factor that influences the lichen flora. All the study sites are not severely affected by traffic related pollution.

Larsen et al. (2007) reported a positive correlation between absence of *Hypogymnia physodes* and the concentration of NO and NO_2 in inner and central London. If the pollution load was high enough, the similar trend could have been observed in this study. However the species was present with occurrence of 100 % at both two roadside sites. This implies that the traffic emission had very little effect on the lichen flora. On the other hand, Gombert et al. (2003) found no influence of urban roads in Grenoble on the nitrogen level of *H. physodes*. The species has low pollution sensitivity (Table 4 & 8) and is considered as pollution-tolerant in Sweden. Therefore it is difficult to determine the impact of pollution from the presence of *H. physodes*, although discolouration of the thalli was observed at Road E4. Monitoring the species by recording the coverage with grid sampling and measuring the nitrogen concentration in thalli can help to understand if the species thrives in their habitat. Bignal et al. (2008) observed the increased growth of some bryophyte species along a motorway in northwest England in relation to the increased nitrogen deposition. Their study was conducted along the motorway that had an average daily flow of 74,000 vehicles, which is almost six times as much as that of E4 (Table 3).

Frati et al. (2006) found no association between epiphytic lichen diversity and NO_2 concentration, probably due to the low concentration of the pollutant in their study. The survey was conducted in a rural area where the maximum NO_2 value was 18 µg/m^3, which is slightly higher than this study. This indicates that the impact would be too small to be detected or to be differentiated from the other factors in this study.

This survey was carried out in forest areas where the ecological condition differs from urban areas. Giordani (2007) points out that the variability of lichen diversity in forest areas is influenced by factors such as harvesting or fires rather than air pollutants. Also, another study found old-growth woodland extent as the major control of epiphyte species richness (Ellis and Coppins, 2009). Even though traffic pollutants affect the lichen flora along the roads to a certain extent, other abiotic and biotic factors as well as forest management can have greater influence on the lichen ecology and may eventually mask the effect of air pollution. Further research requires multiple data analysis including more precise data on each investigated stand.

5. Conclusion

Fewer lichen species recorded at Road E4 than the other sites might be associated with the impact of air pollution. However, other factors such as forestry practices and microclimatic variations in the forest environment may to a large extent influence the lichen flora in this study. On the other hand, the improved air quality at Road 600 may possibly have allowed the lichen recovery. This is the first study to compare the lichen flora along two distinctive roads and there is no previous inventory data. Yet the comparison of the results between Road E4 and Road 600 indicates that the disappearance and recolonisation of lichen species might be fast. Therefore, it is interesting to continue monitoring the lichen diversity and composition every few years to obtain the more solid data regarding the relationship between air quality and lichen flora. More knowledge is required to understand the sensitivity of each species and if there is species-difference in disappearance/recovery rate. With respect to the air quality indices, their accuracy seems to be influenced by various factors such as the selection of trees, the number of observed trees and forestry practice as well as the environmental variables.

Constant increase in the traffic volume implies that air pollution will be present in a long term perspective. Management strategies to assess the impact of traffic emission on the surrounding vegetation are therefore essential. Lichens can be a candidate that signals the rapid change of the environment due to air pollution. It is important to understand how much pollutant level is critical for lichens to disappear or recolonise.

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Appendix
Road 600

Plot/Sample	Coordinates[1]		Altitude (m)	Circumference (cm)	Comments	Bryoria capillaris	Bryoria fuscescens	Cladonia cenotea	Cladonia chlorophaea	Cladonia coniocraea	Cladonia deformis	Cladonia digitata	Cladonia ochrochlora	Cladonia pleurota	Hypocenomyce scalaris	Hypogymnia farinacea	Hypogymnia physodes	Hypogymnia tubulosa	Imshaugia aleurites	Lecidea pullata	Lepraria incana	Mycoblastus sanguinarius	Ochrolechia microsticoides	Permeliopsis ambigua	Permeliopsis hyperopta	Platismatia glauca	Pseudevernia furfuracea	Usnea filipendula	Vulpicida pinastri
	N	E																											
1.1	*	*	54	78	E**, 50 m from										1		1	1	1					1					1
1.2				91	the logging site												1		1					1	1	1	1		
1.3				82													1		1					1	1				
2.1	6701939	632703	62	88	E.										1	1	1		1					1	1	1			
2.2				82						1					1	1	1		1			1		1		1			
2.3				110								1			1		1		1					1		1	1		
3.1	6700834	632745	70	86	E, in front of the					1						1	1							1				1	1
3.2				96	road meeting												1					1		1					
3.3				85	point.											1	1							1					1
4.1	6699378	632689	59	83	E, south of the		1								1		1			1				1	1	1	1	1	1
4.2				79	electric wire.										1		1		1	1				1	1		1	1	1
4.3				82													1			1				1	1	1			1
5.1	6699121	632718	57	95	E, at the curve.										1		1		1	1				1					
5.2				86					1						1		1		1	1				1	1				
5.3				107					1						1		1		1	1				1					1
6.1	6698877	632736	59	81	E, at the curve.					1					1	1	1		1	1				1			1		1
6.2				89											1		1			1				1	1	1	1		1
6.3				86											1	1	1			1				1	1		1		1
7.1	6702284	632623	54	82	W**, young *Pius*												1	1						1	1		1		1
7.2				79	*sylvestris* and *Picea abies*					1							1	1			1			1			1		1
7.3				74	forest.												1	1			1			1			1		1
8.1	6702014	632632	58	79	W, young *Pius*				1	1							1	1						1					1
8.2				83	*sylvestris* and *P.*					1							1							1					1
8.3				79	*abies* forest.					1							1							1					1
9.1	6701567	632650	66	99	W, clear-felled										1		1		1					1		1	1		
9.2				108	on north side of										1		1		1	1		1		1	1	1	1		
9.2				81	plot.										1		1		1					1	1	1	1		
10.1	6700881	632649	63	100	W, sharply							1			1		1			1				1	1				
10.2				97	inclined in a westward							1			1		1			1				1	1				
10.3				94	direction.			1				1		1	1	1	1			1				1	1				
11.1	6699320	632640	58	79	W, south of the												1		1	1				1	1		1		1
11.2				96	electric wire.								1				1			1				1	1				1
11.3				79													1		1	1				1					1
12.1	6699056	632660	56	107	W, at the curve.			1					1		1		1	1	1	1				1	1		1		1
12.2				92						1	1			1			1						1	1	1				1
12.3				87											1		1		1	1				1	1	1	1	1	

[1] The geographic coordinate system is based o SWEREF 99 ad the coordinates were marked at the centre of each quadrat.

*****Geographic coordinates for the plot 1 at Road 600 are missing.

******E = East side of the road, W = West side of the road

Road E4

Plot/Sample	Coordinates		Altitude (m)	Circumference (cm)	Comments	Bryoria capillaris	Bryoria fuscescens	Cladonia cenotea	Cladonia chlorophaea	Cladonia coniocraea	Cladonia deformis	Cladonia digitata	Cladonia ochrochlora	Cladonia pleurota	Hypocenomyce scalaris	Hypogymnia farinacea	Hypogymnia physodes	Hypogymnia tubulosa	Imshaugia aleurites	Lecidea pullata	Lepraria incana	Mycoblastus sanguinarius	Ochrolechia microsticoides	Permeliopsis ambigua	Permeliopsis hyperopta	Platismatia glauca	Pseudevernia furfuracea	Usnea filipendula	Vulpicida pinastri
	N	E																											
1.1	6687737	639196	57	89	E, at the end of												1		1	1				1	1				1
1.2				80	the ditch along												1		1	1				1					1
1.3				84	the fence.			1									1		1	1				1	1				1
2.1	6687470	639136	60	85	E, north end of				1								1			1	1	1		1	1				1
2.2				100	the hill												1							1					1
2.3				92													1		1	1				1	1				1
3.1	6687239	639071	60	91	E, end of the												1		1	1				1					
3.2				90	hill. Inclined												1		1	1				1	1				1
3.3				106	northwards.												1			1			1	1					
4.1	6687033	638982	62	99	E, on the hill.												1		1	1				1					1
4.2				93													1							1	1				1
4.3				87										1			1							1	1				1
5.1	6686817	638852	61	86	E, on the hill.											1	1			1	1			1	1		1		1
5.2				91												1	1			1	1			1		1			
5.3				85													1		1	1				1					
6.1	6686377	638633	60	102	E, *P. abies*										1		1			1	1			1	1				1
6.2				84	forest on south			1									1		1	1				1					1
6.3				81	of the plot.												1		1	1	1			1	1				1
7.1	6688198	639388	61	89	W, north of the												1			1				1					1
7.2				80	electric wire.												1			1				1					1
7.3				79													1							1					1
8.1	6687957	639384	64	99	W, just south of				1								1			1				1					1
8.2				90	the electric wire.				1								1							1					1
8.3				99													1							1	1				1
9.1	6687738	639349	64	79	W, 100 m south				1								1			1				1	1				1
9.2				93	of the resting												1			1				1					1
9.2				92	place and swamp.			1	1					1			1			1	1			1					1
10.1	6687518	639293	61	83	W, south of the				1								1			1	1		1	1	1				
10.2				86	slightly elevated			1									1			1			1	1	1				1
10.3				91	hill.												1			1				1	1				
11.1	6686973	639073	64	103	W, open area.										1		1			1	1	1		1					
11.2				101								1					1			1	1			1					
11.3				91												1	1			1	1			1					
12.1	6686749	638955	62	84	W, just before												1	1		1	1			1		1			1
12.2				80	the tunnel that go under the												1			1				1					1
12.3				80	motorway.												1			1				1					1

Reference site

Plot/Sample	Coordinates		Altitude (m)	Circumference (cm)	Comments	Bryoria capillaris	Bryoria fuscescens	Cladonia cenotea	Cladonia chlorophaea	Cladonia coniocraea	Cladonia deformis	Cladonia digitata	Cladonia ochrochlora	Cladonia pleurota	Hypocenomyce scalaris	Hypogymnia farinacea	Hypogymnia physodes	Hypogymnia tubulosa	Imshaugia aleurites	Lecidea pullata	Lepraria incana	Mycoblastus sanguinarius	Ochrolechia microsticoides	Permeliopsis ambigua	Permeliopsis hyperopta	Platismatia glauca	Pseudevernia furfuracea	Usnea filipendula	Vulpicida pinastri
	N	E																											
1.1	6686529	629118	40	95	10 m south of the gravel road. Clear-felling on west side of plot.			1	1			1					1		1	1				1	1				
1.2				109				1											1		1			1	1				
1.3				104				1				1					1		1					1	1				1
2.1	6686336	629157	40	87	Clear-felling on west side of plot.			1						1					1	1				1	1				
2.2				84				1		1							1		1	1				1	1				1
2.3				101						1					1		1			1				1	1				
3.1	6686115	629142	40	81	Clear-felling on west side of plot.					1				1			1		1	1				1	1				1
3.2				92											1		1			1	1			1	1				
3.3				84					1								1		1	1				1	1				1
4.1	6685887	628873	40	85	*Betula* spp. forest on west side of plot.	1	1										1			1				1					
4.2				103		1											1			1				1					
4.3				108		1									1		1							1				1	1
5.1	6685604	628870	40	82	Young *P. abies* forest on east side of plot.	1		1		1		1			1		1			1				1	1			1	
5.2				110		1				1					1		1			1				1	1				
5.3				98							1				1		1			1				1					1
6.1	6685360	628821	40	97	*Betula* spp. forest on east side of plot.			1	1	1	1	1					1			1	1			1	1			1	1
6.2				99				1		1	1						1			1				1	1				
6.3				109				1	1	1										1	1			1	1				1
7.1	6685062	629074	40	103	Close to the logging road.					1			1	1			1			1				1	1				1
7.2				103						1				1			1							1	1				1
7.3				108					1	1							1			1	1			1	1	1			1
8.1	6684759	629102	40	93	Logging road on north and east side of plot.										1		1			1	1			1	1				
8.2				104											1		1			1	1			1					
8.3				110											1		1			1	1			1					
9.1	6684551	629147	40	103	Many species found on braches of *P. abies* ad *P. sylvestris.*												1			1	1			1	1	1			1
9.2				84				1									1			1				1				1	1
9.2				88													1			1				1	1				1
10.1	6684435	629422	40	86	Close to the logging road, clear-felled on south side of plot.							1	1		1		1			1	1			1					
10.2				109				1									1			1	1			1	1				1
10.3				99				1				1				1	1			1			1	1	1				
11.1	6684191	629462	40	106	Close to the logging road, clear-felling on south side of plot.			1							1		1			1				1	1				1
11.2				94									1				1			1				1	1				1
11.3				83				1	1								1			1	1			1	1				1
12.1	6684795	629256	40	82	Close to the logging road.									1			1			1	1			1					1
12.2				86								1					1			1				1					1
12.3				87				1	1				1				1			1				1					1